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CONTRACTING ORGANIZATION: University of Maryland at Baltimore
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13. ABSTRACT *(Maximum 200*

Nausea and vomiting are severe side-effects often associated with cancer chemotherapy and may affect treatment decisions. In order to examine the effects of electro-acupuncture (EA) on the emetogenic effect of cyclophosphamide a commonly used chemo-therapy agent for breast cancer, ferrets (1.0-2.0 kg) were placed under general anesthesia (isoflurane 5%-oxygen mixture) and were administered logarithmic doses of i.v. cyclophosphamide. A dose of 177 mg/kg produced the maximal number of emetic episodes (23.3 ± 4.0 episodes) with an emetic profile consisting of two phases (first phase 18.8 ± 3.9 episodes;second phase 4.7 ± 1.2 episodes). For treatment, EA was given under general anesthesia followed by i.v. cyclophosphamide (177mg/kg). Various parameters were evaluated and the results indicated that EA (100Hz,1.5V,10 min) effectively treated the first emetic phase induced by cyclophosphamide (9.3 ± 1.8 episodes for the first phase), an effect similar to the antiemetic drug ondansetron. Studies using combination therapy of EA with ondansetron (0.04mg/kg) or metoclopramide (2.24mg/kg) showed a significant reduction in the total number of emetic episodes ($p\leq0.05$;$p\leq0.005$ respectively). This indicates that EA may be useful as an adjunctive therapy in the treatment of chemotherapy-induced emesis

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FOREWORD

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Li Lao 7-22-94

PI - Signature Date

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FINAL REPORT

Principal Investigator: Lixing Lao, Ph.D., L.Ac.

Grant Number DAMD17-94-J-4325

Title: The Effects of Electroacupuncture on Cyclophosphamide-Induced Emesis in Ferrets

Prepared for: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

INTRODUCTION

Nausea and vomiting (N/V) are common incidences among patients who have cancer chemotherapy (Coates et al., 1983; Watcha & White, 1992). Antiemetic drugs do not completely block N/V and most often add to the unpleasant effects of treatment (Cubeddu et al., 1990b; D'Olimpia et al., 1985; Watcha & White, 1992). Among the various treatment modalities to reduce N/V, the effect of acupuncture point P6 has been investigated in clinical trials (Aglietti et al., 1990; Dundee, 1991). The clinical studies by Dundee's group indicated that invasive acupuncture combined with antiemetic drug therapy benefited cancer patients in chemotherapy which included cyclophosphamide (Dundee et al., 1989). Other studies conducted by Dundee's group showed that acupressure and transcutaneous electrical stimulation (TENS) of the same acupoints also benefited the patient undergoing chemotherapy (Dundee & Yang, 1990; Dundee et al., 1991). Aglietti's group demonstrated that acupuncture effectively decreased N/V in patients treated with cisplatin (Aglietti, et al., 1990).

Cyclophosphamide is a commonly used agent in chemotherapy for breast cancer and induces emesis in a ferret model (Andrews et al., 1988; Hawthorn et al., 1988). Cyclophosphamide may induce emesis through release of serotonin to stimulate the 5-HT_3 receptor in the gastrointestinal tract and the chemoreceptor trigger zone (Fraschini et al., 1991; Hawthorn et al., 1988). The 5-HT_3 antagonists such as ondansetron have been shown to be moderately effective antiemetics for cyclophosphamide-induced emesis in ferrets (Andrews et al., 1988) and humans (Clavel et al., 1993; Cubeddu et al., 1990a; Fraschini et al., 1991; Rosso et al., 1991). Side effects have included headache, light-heatedness and transient elevations of hepatic transaminases (Clavel et al., 1993; Cubeddu et al., 1990a; Einhorn et al., 1990; Fraschini et al., 1991; Hesketh & Gandara, 1991; Rosso et al., 1991). The combination dopamine/5-HT_3 antagonist metoclopramide has been moderately effective in reducing cyclophosphamide-induced emesis in humans (Clavel et al., 1993). Metoclopramide has been shown to produce adverse extrapyramidal side effects in humans (Sanger, 1990). There is no animal model to study the antiemetic effects of acupuncture, however, our pilot study showed that EA given at acupuncture point P6 reduced morphine-induced emesis by 39-43% (Lao et al., 1995).

Acupuncture has been used to treat a variety of diseases, including pain, in China for thousands of years. According to Traditional Chinese Medicine (TCM), there are 12 primary channels or meridians and 8 additional meridians, each following a directional course along the body. A vital energy known as *Qi* flows through these meridians and participates in the homeostatic regulation of various body functions. Some 360 points distributed along the meridians serve as both pathognomic signs of disorder and as loci for acupuncture treatments (O'Connor & Bensky, 1981; Stux & Pomeranz, 1987). The meridian flow of *Qi* can be blocked by any pathogen which results in various symptoms or syndromes. Accordingly, acupuncture treatment involves the insertion of small-gauge needles into specific points as indicated by the nature of the imbalance in order to restore the vital flow of energy through affected meridians (O'Connor & Bensky, 1981; Stux & Pomeranz, 1987). The needles are typically left in place for 20-30 minutes. The effects of acupuncture may be augmented with electrical stimulation (EA) and/or heat (e.g. moxibustion). Side-effects from acupuncture are rare and tend to be associated with violations of sterile procedure and/or negligence on the part of the acupuncturist (Kent & Brondum, 1988; Wright et al., 1991).

A pilot study in our laboratories has shown that the acupuncture technique can be transferred to the ferret by modification of the acupuncture points in humans (Lao et al., 1995). This study showed that EA significantly reduced the number of emetic episodes induced by morphine (Lao et al., 1995). In humans, the acupuncture point Neiguan (P6) is located on the forearm, 2 units directly above the midpoint of the transverse crease of the wrist (the distance between cubital and carpal creases is 12 units), between the tendons of the flexor carpi radialis and the palmaris longus muscles. Below this point is the median nerve (O'Connor & Bensky, 1981). The equivalent point in ferrets had been located in our pilot study (Lao et al., 1995).

The specific aims of the present study are:

1. To determine the emetogenic effect of cyclophosphamide in the ferret.
2. To determine the most effective EA conditions and to evaluate the effect of EA in reducing cyclophosphamide-induced emesis in the ferret.
3. To test the antiemetic effects of ondansetron, metoclopramide, and droperidol against cyclophosphamide-induced emesis in the ferret and to compare these effects to EA.
4. To evaluate that an EA-drug combination is more efficacious as an antiemetic against cyclophosphamide in the ferret than either treatment alone.

BODY

Ferrets were castrated males, 1.0-2.0 kg in weight and from the Triple F Farm, Sayre, PA. Ferrets were made unconscious by general anesthesia (Isoflurane 5%-O_2 mixture) to restrain them for acupuncture treatment. For testing, ferrets were anesthetized with isoflurane 5%-O_2 mixture while contained in a 20 gallon glass aquarium box with a removable plastic cover. The anesthetic gas was delivered from a vaporizer (Fortec), calibrated for isoflurane, through polyethylene tubing into the box and was scavenged out using a vacuum tubing vented to the outside air. Each ferret was removed after loss of righting (2-5 min) and immediately weighed. For EA, each animal was maintained under isoflurane 2.5%-O_2 anesthesia delivered from a second vaporizer through a small nose cone. For EA treatment, the equivalent acupuncture point P6 in the ferret was located at the forepaws (Lao et al., 1995). After needle insertion (disposable needle, gauge # 34, diameter 0.22 mm, length 1 in., depth of 0.3-0.5 in.), the stimulator's electrodes (Grass) were attached to the end of the needles and electrical stimulation was applied (the EA parameters will be described in detail later). The frequency and voltage of stimulation were monitored by an oscilloscope (Tektronix).

Specific Aim #1.

Ferrets were given i.v. cyclophosphamide at log doses of 56, 100, 177, and 237 mg/kg (n=6 for all doses except 237 mg/kg where n=2). For the i.v. route of administration, cyclophosphamide injections were made into the cephalic vein on the dorsal aspect of a front paw using a rubber tourniquet and a 3 or 5 ml syringe with a 25 G needle while the ferret was under general anesthesia. The forepaw was shaved for ease of vein location. Intravenous puncture was confirmed by aspirating a small volume of blood into the syringe and injections confirmed by lack of resistance to the syringe plunger. After injection, each ferret was placed into an individual compartment (60 x 60 x 38 cm^2) of a cage rack holding six compartments having wire mesh floors elevated to the height of door threshold and modified with a plexiglass front door for ease of viewing. Complete recovery from anesthesia occurred in all ferrets within 3-10 min. Emetic action of the animal was observed and the onset time of emesis was recorded. The number of episodes of retching and vomiting were also recorded along with the prodromal signs of nausea: salivation, head shake, lip lick, walking backwards, posturing, sedation, and slit eyes (Wynn et al., 1993). Statistical analysis was done using Student's two-way t-test with a $p \leq 0.05$ considered significant. After each experiment, the ferrets were sacrificed using carbon dioxide (CO_2). This study (amended 11/29/93) has been approved by the Institutional Animal Care and Use Committees (Ref. #134200-039301 and #93-04-01) at the School of Medicine and the Dental School, University of Maryland at Baltimore.

The results indicated that the dose of 177 mg/kg produced the maximal number of emetic episodes (23.3±4.0 emetic episodes; Table 1; Appendix; Wong et al., 1996). The dose of 237 mg/kg was not chosen for further experiments since it had toxic effects. Cyclophosphamide induced emesis in a dose-dependent manner producing two distinct emetic phases that were separated by a one hour time period (Fig. 1). The first phase resulted in a mean of 18.6±3.9 emetic episodes and the second phase produced 4.7±1.2 emetic episodes. These two phases were used to compare the effects of EA, antiemetic drug therapy, and combination therapy.

Table 1. Emetogenic Effect of Cyclophosphamide by Dose in Ferrets

Dose (mg/kg)	*No. Vomiting/N*	*Mean±S.E. Emetic Episodes*	*Mean±S.E. Retches*
Cyclophosphamide			
56	4/6	2.2±0.9	2.8±1.9
100	5/6	7.3±3.2	30.5±17.5
177	8/8	23.3±4.0	85.3±20.4
237	2/2	23.5±7.5	62.5±38.5

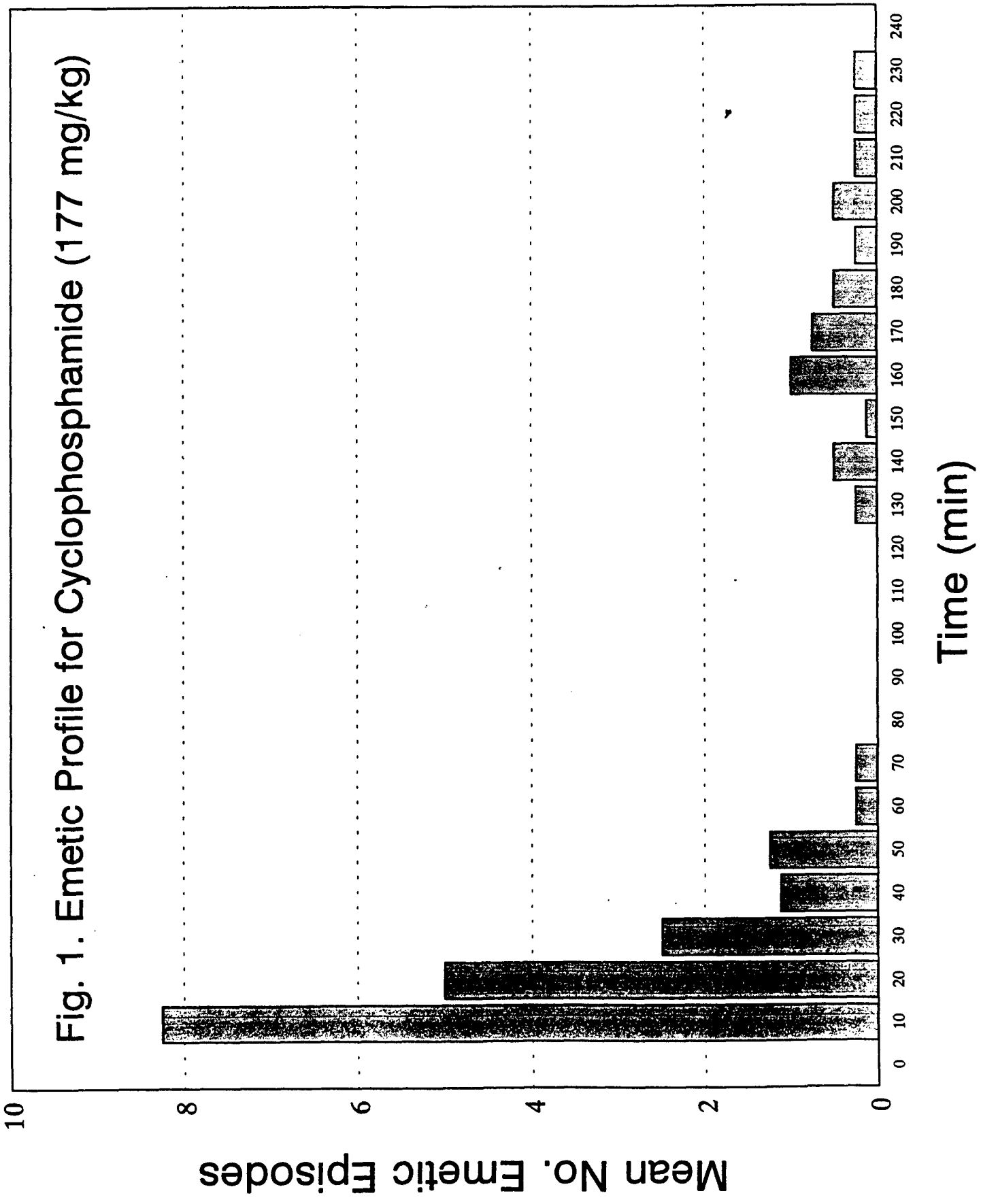
Fig. 1. Emetic Profile for Cyclophosphamide (177 mg/kg)
Mean No. Emetic Episodes
0
2
4
6
8
10
Time (min)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240

Specific Aim #2.

Changes in the frequency (5 and 100Hz), intensity (1.5 and 3V), and duration (5, 10, and 20 min) of electrical stimulation were evaluated (n=6/group; Table 2, Figs. 2-6). EA was administered followed immediately by i.v. cyclophosphamide (177 mg/kg). Our results indicated that EA at 100 Hz, 1.5V, 10 min produced the most beneficial antiemetic effect as compared to other parameters. Using these stimulation parameters, EA was more effective against the first emetic phase with a mean of 9.3±1.8 emetic episodes (Table 2, Fig. 2). This resulted in a 50% decrease in emetic episodes for the first phase. For all tested parameters, there was no significant reduction in the total number of emetic episodes as compared to control.

Table 2. Electroacupuncture Against Cyclophosphamide in Ferrets

EA Parameters (Frequency, Intensity, Duration)	*N*	*Mean±S.E. Emetic Episodes*	
		First Phase[1]	*Second Phase[2]*
Vehicle	8	18.6±3.9	4.7±1.2
5Hz, 3V, 10 min	6	10.3±1.9	10.7±2.5*
100Hz, 1.5V, 20 min	6	16.5±4.6	8.3±1.1*
100Hz, 1.5V, 5 min	6	11.8±4.3	8.7±0.9*
100Hz, 3.0V, 10 min	6	13.2±5.3	7.7±2.0
100Hz, 1.5V, 10 min	6	9.3±1.8	10.0±2.5
Sham	6	14.0±2.7	7.8±1.4
Placebo	6	12.2±3.0	10.5±1.7*

(*$p \leq 0.05$ compared to vehicle control values; [1] Time 0 to 70 min; [2] Time 130 to 240 min)

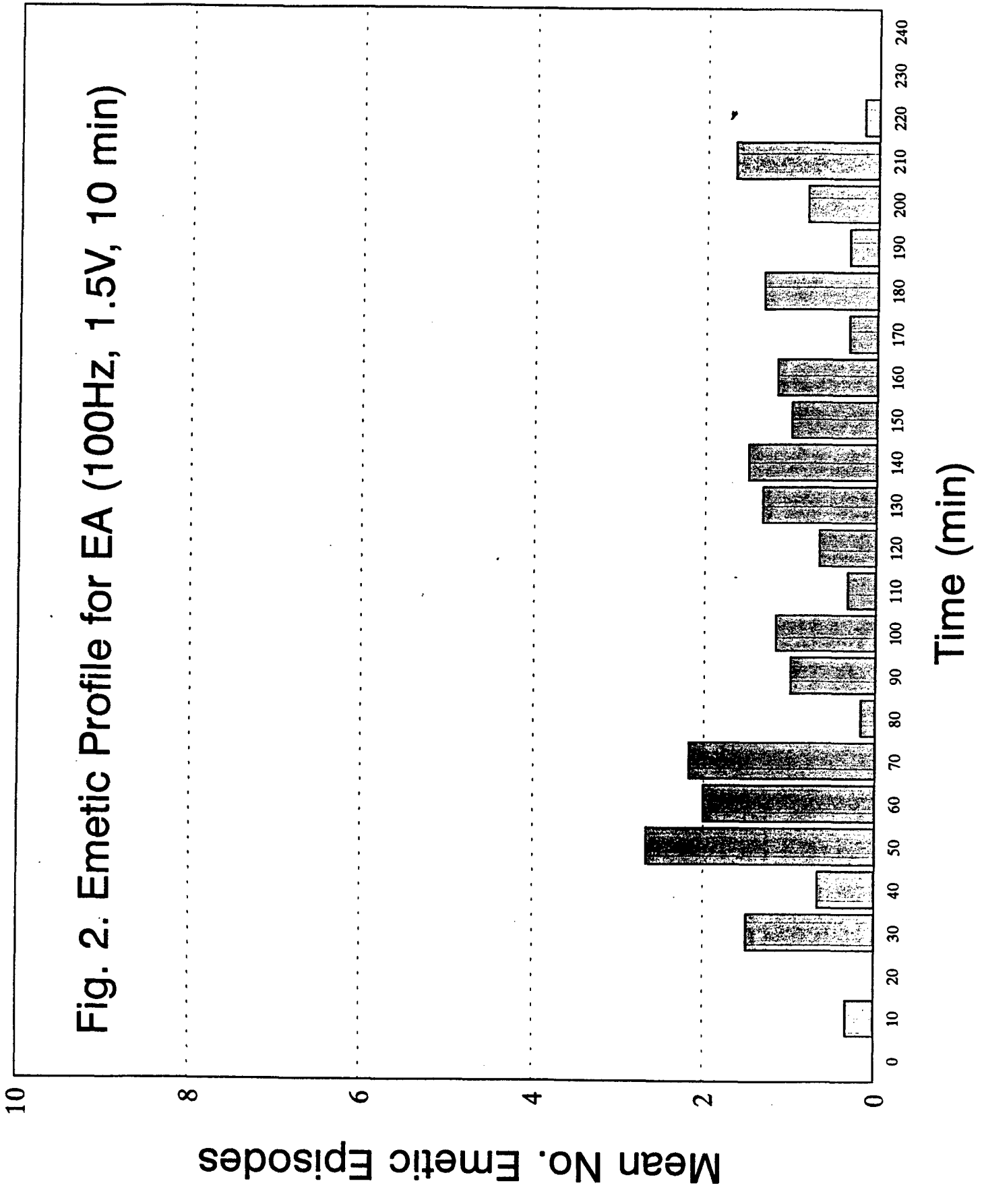
Fig. 2. Emetic Profile for EA (100Hz, 1.5V, 10 min)
Mean No. Emetic Episodes
0
2
4
6
8
10
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

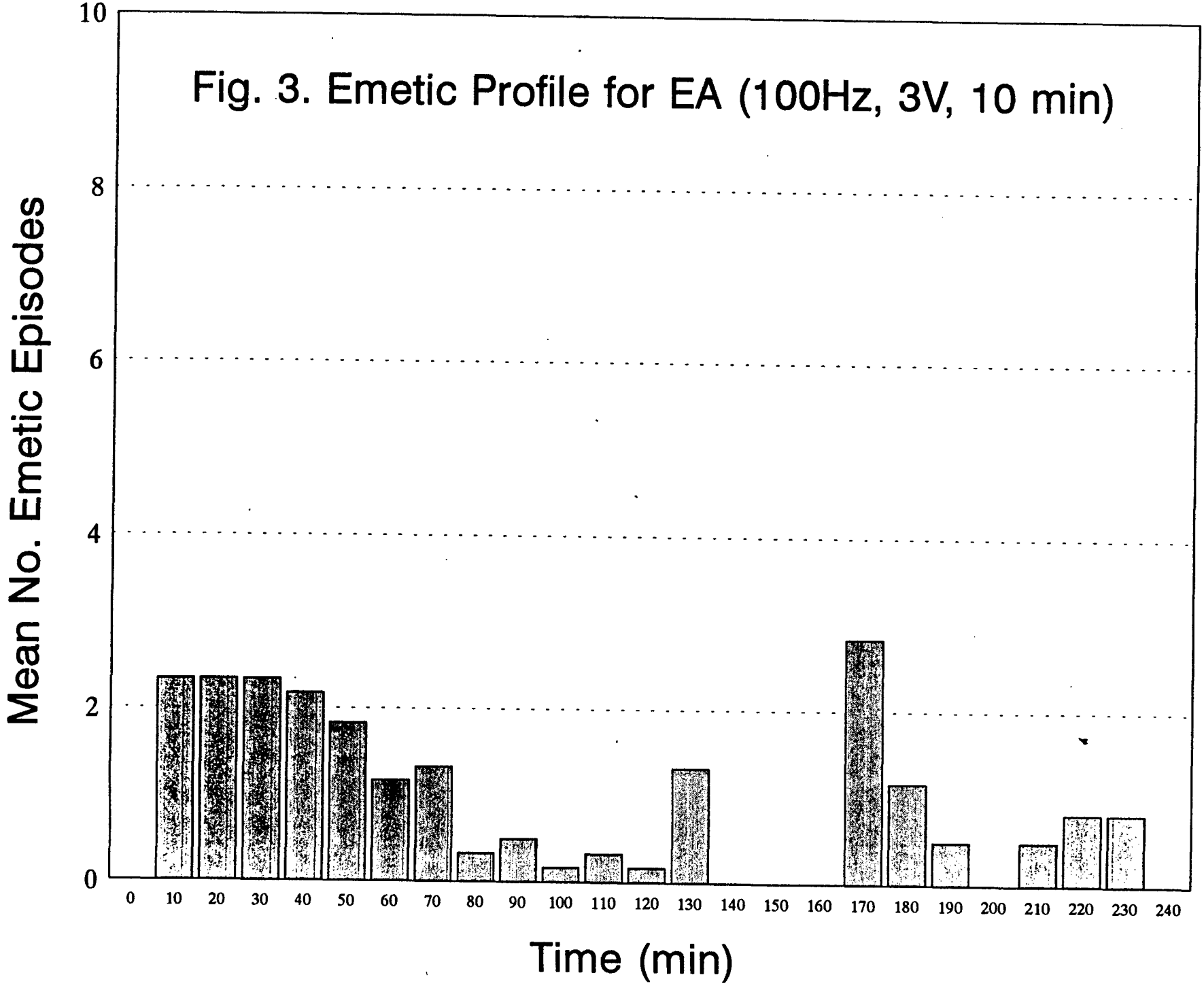
Fig. 3. Emetic Profile for EA (100Hz, 3V, 10 min)
Mean No. Emetic Episodes
0
2
4
6
8
10
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

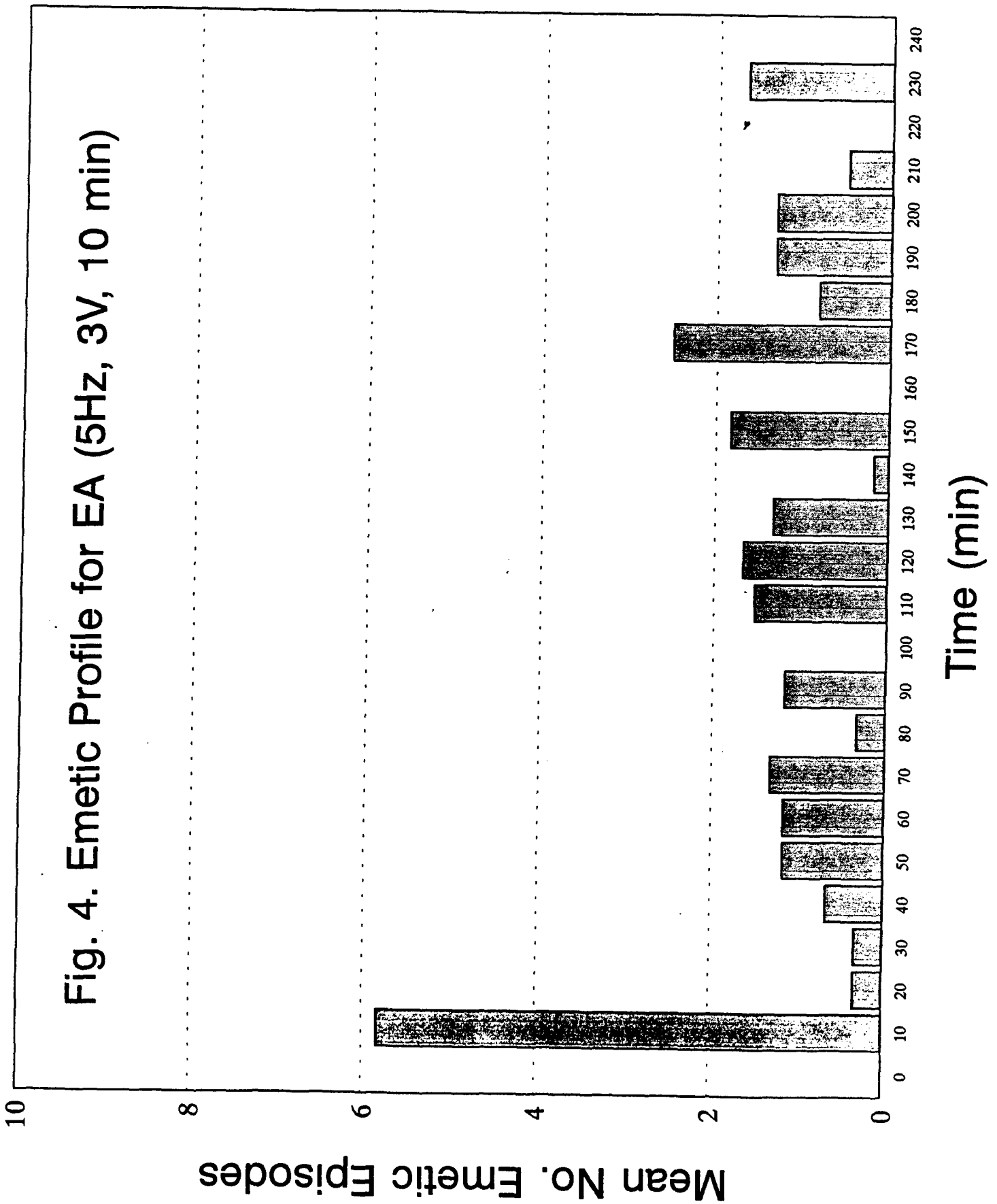
Fig. 4. Emetic Profile for EA (5Hz, 3V, 10 min)
Mean No. Emetic Episodes
0
2
4
6
8
10
Time (min)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240

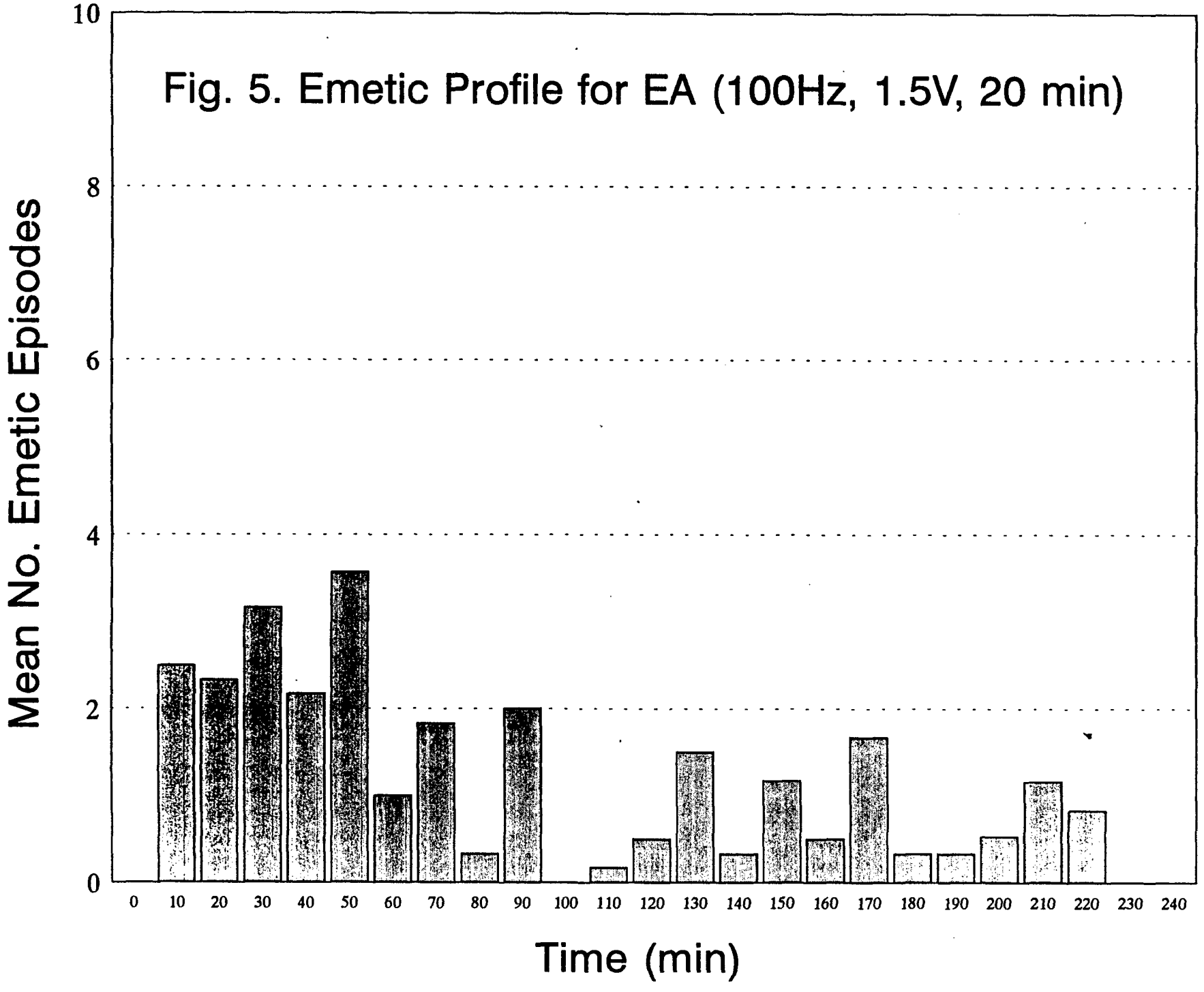
Fig. 5. Emetic Profile for EA (100Hz, 1.5V, 20 min)
Mean No. Emetic Episodes
0
2
4
6
8
10
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

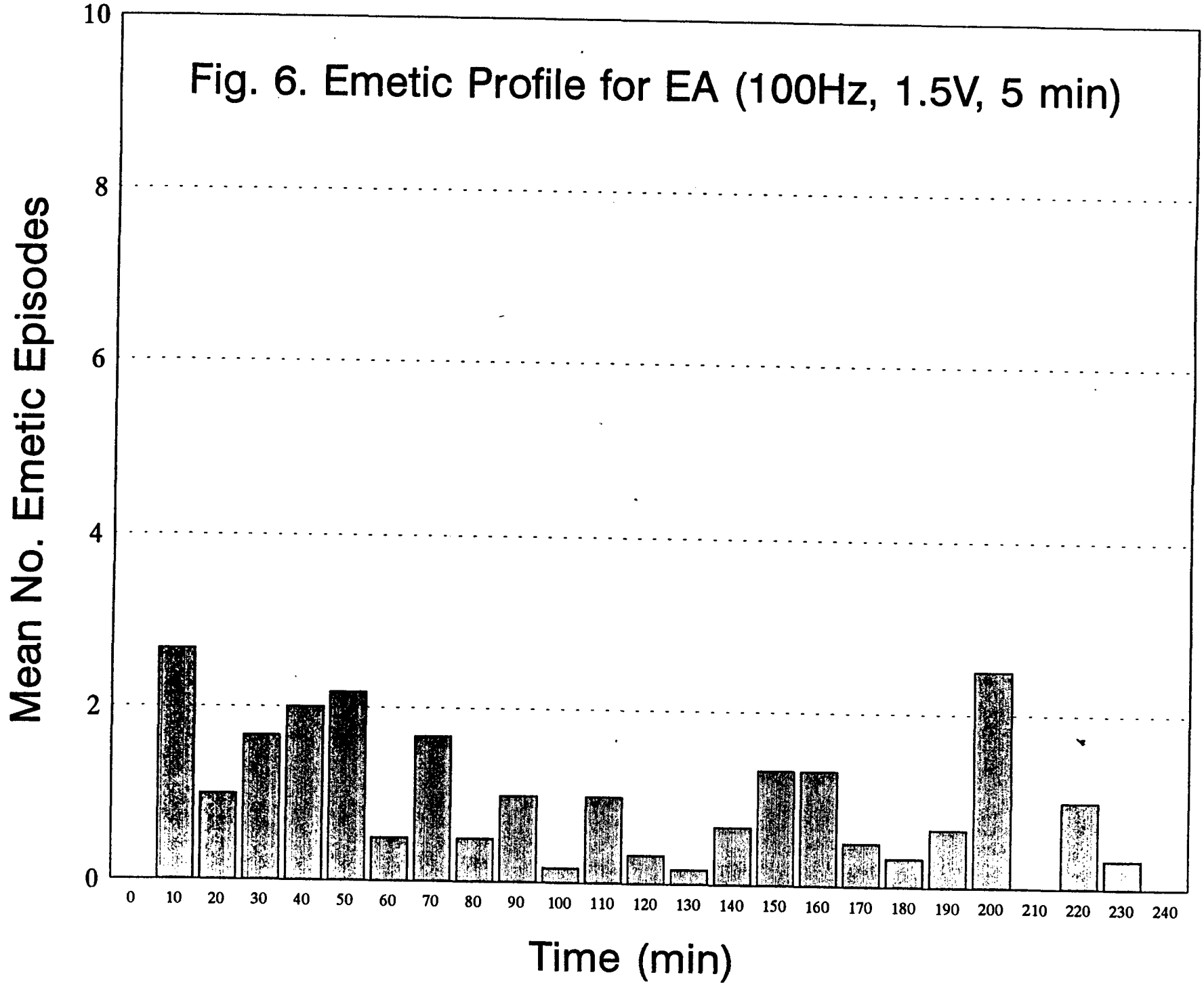
Fig. 6. Emetic Profile for EA (100Hz, 1.5V, 5 min)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

After the evaluation of the EA parameters, controlled experiments were done to compare the effectiveness of EA (100Hz, 1.5V, 10 min) against cyclophosphamide-induced emesis with respect to sham acupuncture and non-treatment. In the sham acupuncture group, a non-acupuncture point (dummy point) was used at the elbow area (Dundee, 1991), and no electrical stimulation was applied. The results indicated a mean first phase of 14.0±2.7 emetic episodes (Table 2, Fig. 7). In the placebo (non-treatment) group, animals received the same protocol as the acupuncture group except that the acupuncture needles were taped on the skin of the animal (rather than inserted) and did not have electrical stimulation. This resulted in a mean first phase of 12.2±3.0 emetic episodes (Table 2, Fig. 8). No statistical significance among these three groups (EA vs Sham: $p=0.1$; EA vs Placebo: $p=0.2$). However, the results showed trend that EA group has lowest episodes in the first phase of emesis induced by cyclophosphamide compared to sham and placebo. In order to assess any adverse effects associated with this type of therapy, animals were treated with acupuncture alone (n=6) without injection of cyclophosphamide. Any unusual animal behavior changes would be observed, recorded and analyzed, such as seizures, dry mouth, salivation, moving head back and forth, fine tremor, or sedateness. None of these unusual behaviors were observed from the EA treatment.

Specific Aim #3.

Three antiemetic drugs were used for the treatment of cyclophosphamide-induced emesis: ondansetron, metoclopramide, and droperidol. Using log doses, the antiemetic drugs were administered i.v. immediately following cyclophosphamide injection (177 mg/kg). Ondansetron reduced emetic episodes by 0, 42, and 9% (0.04, 0.07, and 0.13 mg/kg) (Table 3, Figs. 9-11; Wong et al., 1996). This drug produced an emetic profile similar to acupuncture in which it was able to effectively treat the first phase of emesis but increased the number of episodes in the second phase. Metoclopramide reduced emetic episodes by 48, 65, and 98% (2.24, 4.08, 7.07 mg/kg) (Table 3, Figs. 12-14; Wong et al., 1996). Metoclopramide significantly reduced the number of emetic episodes in the first phase and completely prevented emesis in the second phase at a dose of 7.07 mg/kg. Droperidol resulted in a 24, 16, and 38% reduction (0.25, 0.45, 0.79 mg/kg) in which it was able to significantly reduce the first emetic phase but increased the number of emetic episodes in the second phase (Table 3, Fig. 15-17).

Specific Aim #4.

Combination therapy was evaluated in ferrets (n=6) who were first treated with EA (100 Hz, 1.5V, 10 min) since it was effective against the first emetic phase. This was followed by injection with cyclophosphamide (177 mg/kg). The antiemetic drug was then given i.v. immediately following cyclophosphamide. Ondansetron (0.04 mg/kg) combined with EA significantly reduced the total number of emetic episodes (11.8±2.2) and the number of emetic episodes in the first phase (Table 4). Ondansetron at a dose of 0.02 mg/kg significantly increased the second emetic phase (Table 4). EA and metoclopramide (2.24 mg/kg) significantly reduced the total number of emetic episodes (6.2±2.0). Metoclopramide at this dose and also at a lower dose of 1.26 mg/kg significantly reduced the number of emetic episodes in the first phase (Table 4). Combination of EA with droperidol (0.25 and 0.45 mg/kg) significantly reduced the number of emetic episodes in the first emetic phase in which it significantly increased the second phase at the dose of 0.45 mg/kg (Table 4).

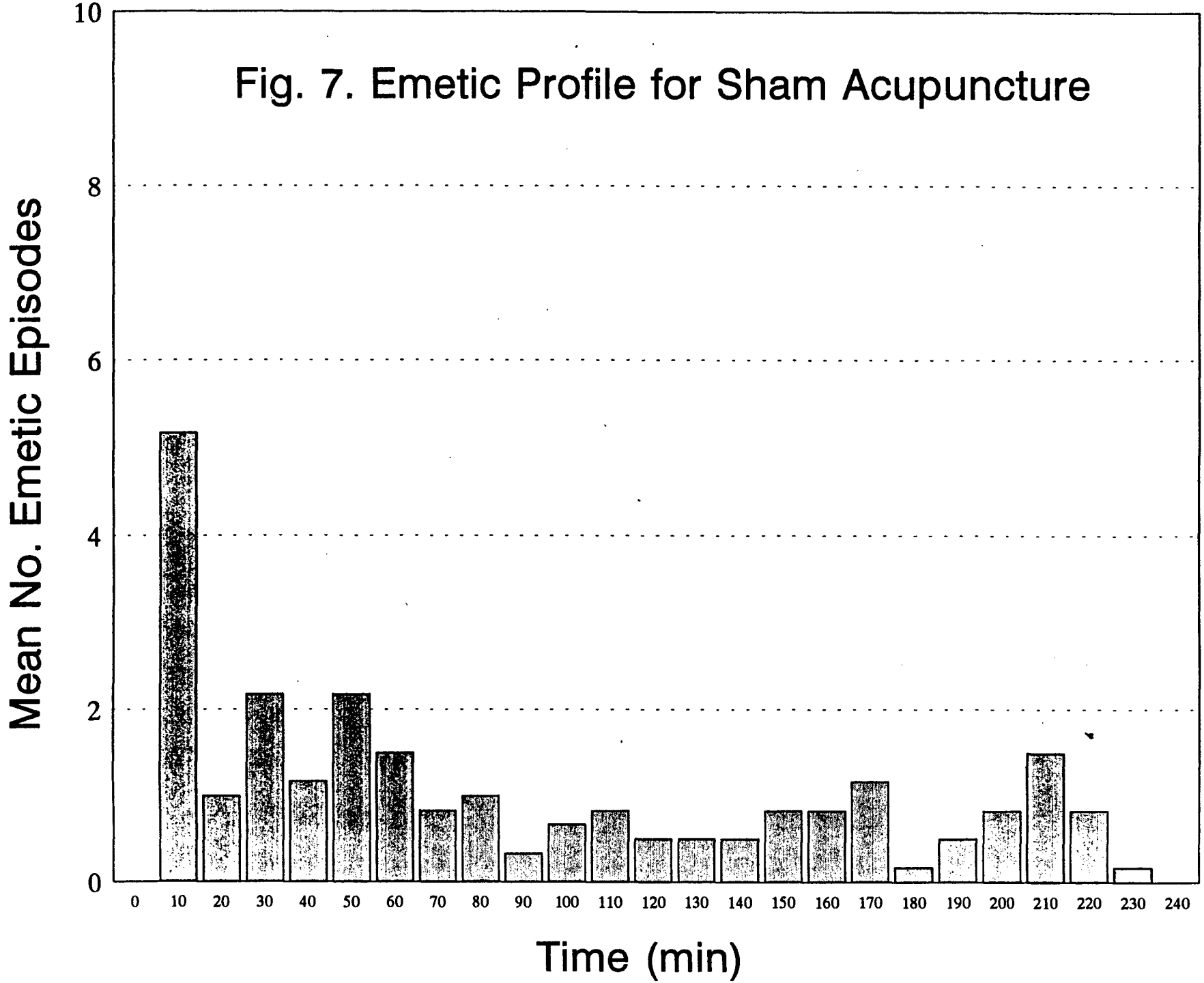
Fig. 7. Emetic Profile for Sham Acupuncture
Mean No. Emetic Episodes
Time (min)
0
2
4
6
8
10
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240

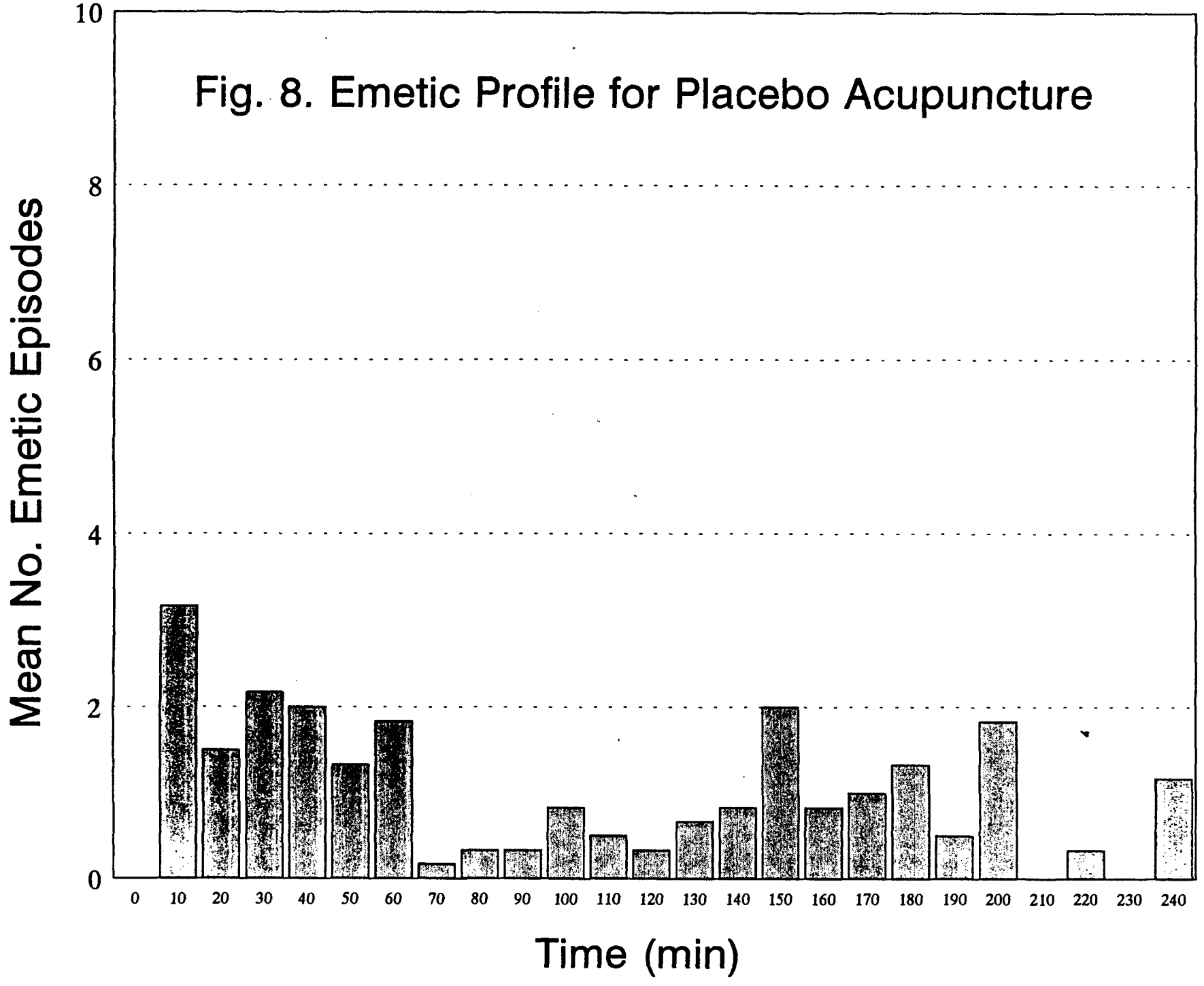
Fig. 8. Emetic Profile for Placebo Acupuncture
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

Table 3. Dose Response of Antiemetics Against Cyclophosphamide in Ferrets

Dose (mg/kg)	*N*	*Mean±S.E. Emetic Episodes*	
		First Phase[1]	*Second Phase*[2]
Vehicle	8	18.6±3.9	4.7±1.2
Ondansetron			
0.04	5	12.2±3.7	15.4±2.8**
0.07	5	0.4±0.4**	11.0±2.1*
0.13	5	2.8±1.7*	18.2±3.5**
Metoclopramide			
2.24	5	3.2±2.7*	8.2±3.1
4.08	5	3.6±1.9*	3.8±1.3
7.07	5	0.4±0.2**	0.0±0.0
Droperidol			
0.25	5	5.8±2.6*	11.4±2.7*
0.45	5	12.0±2.3	6.8±2.2
0.79	5	5.6±3.1*	8.8±1.7

(*p≤0.05, **p≤0.005 compared to vehicle control values; [1] Time 0 to 70 min; [2] Time 130 to 240 min)

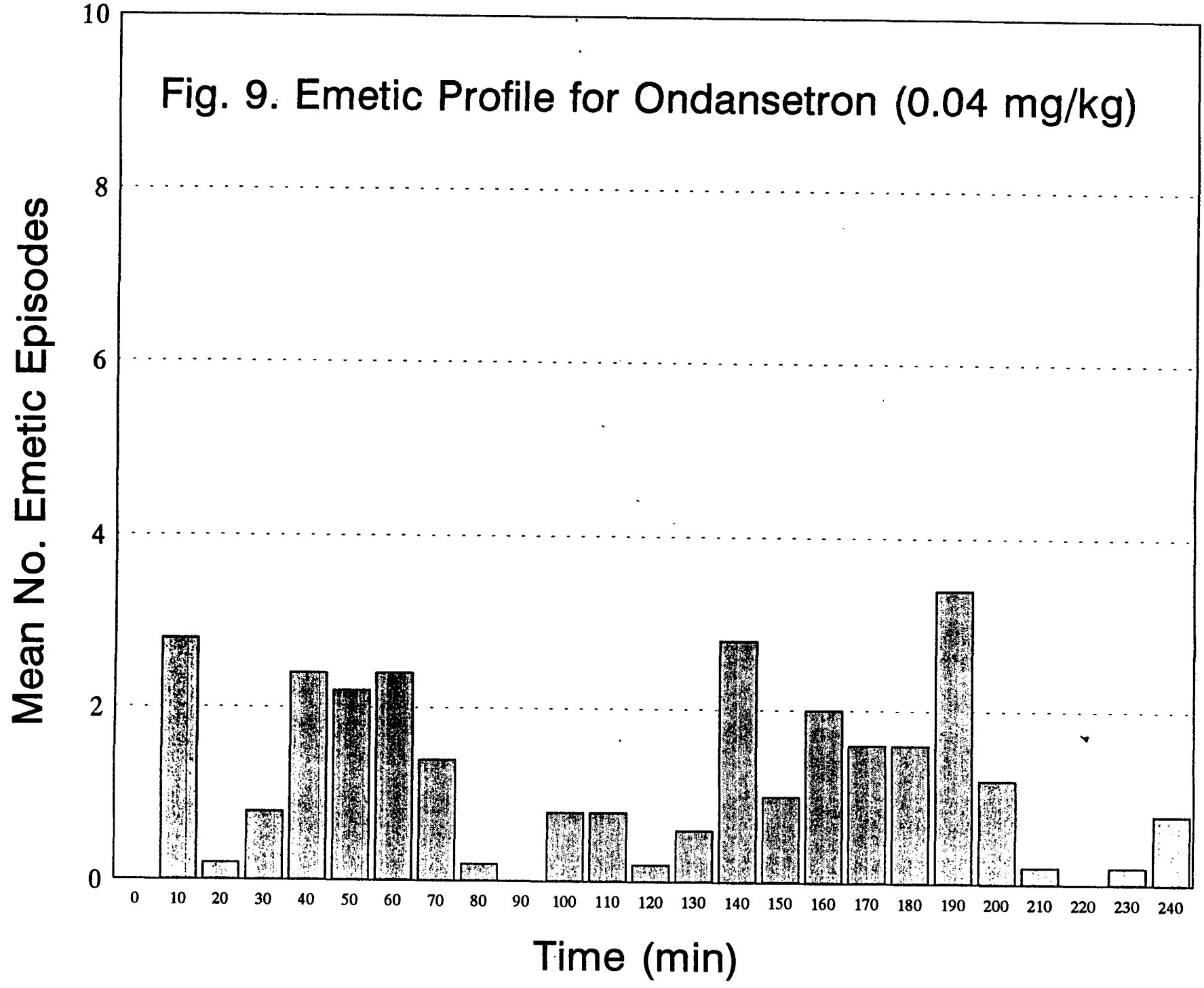
Fig. 9. Emetic Profile for Ondansetron (0.04 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

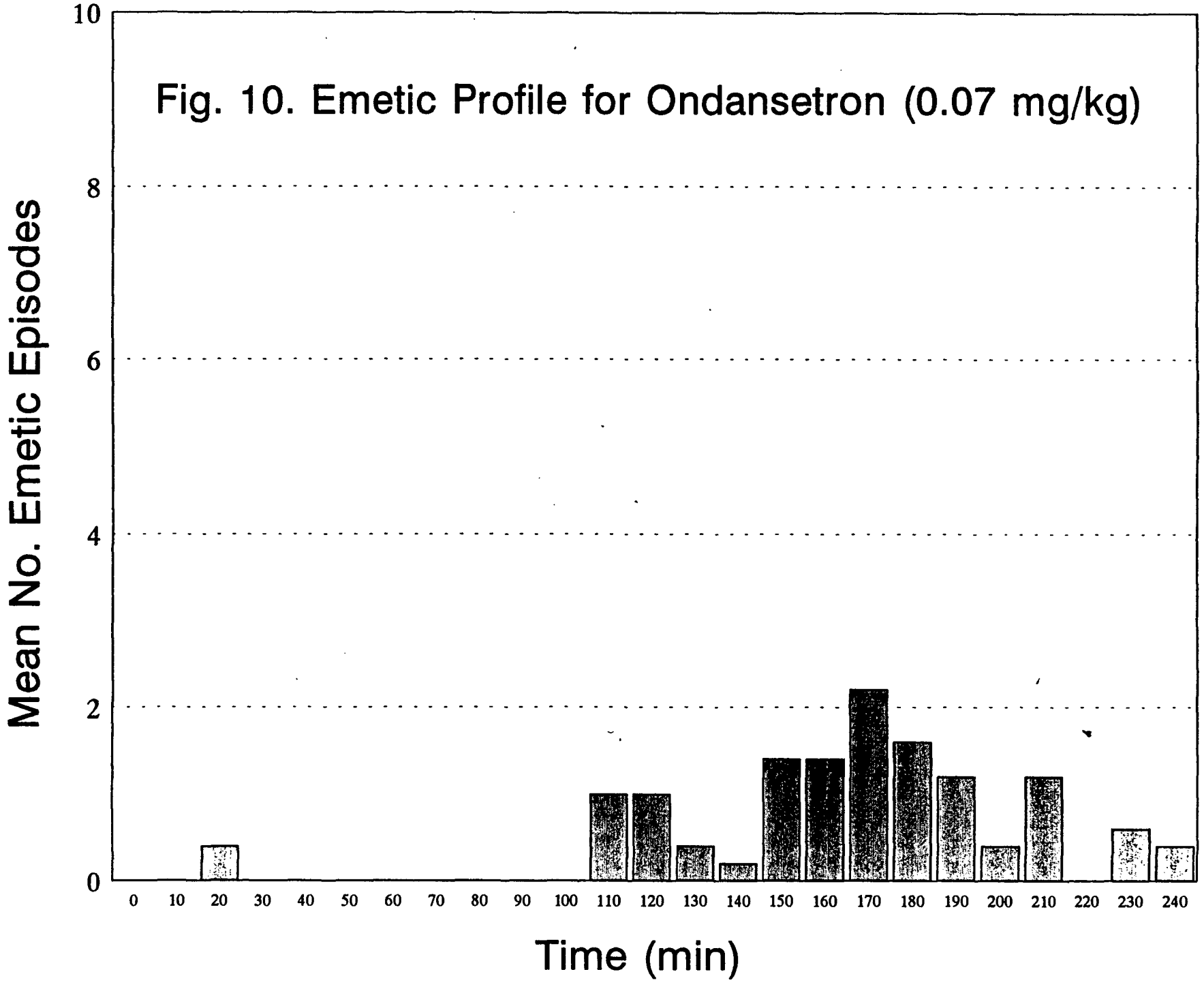
Fig. 10. Emetic Profile for Ondansetron (0.07 mg/kg)
Mean No. Emetic Episodes
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2
4
6
8
10
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Time (min)

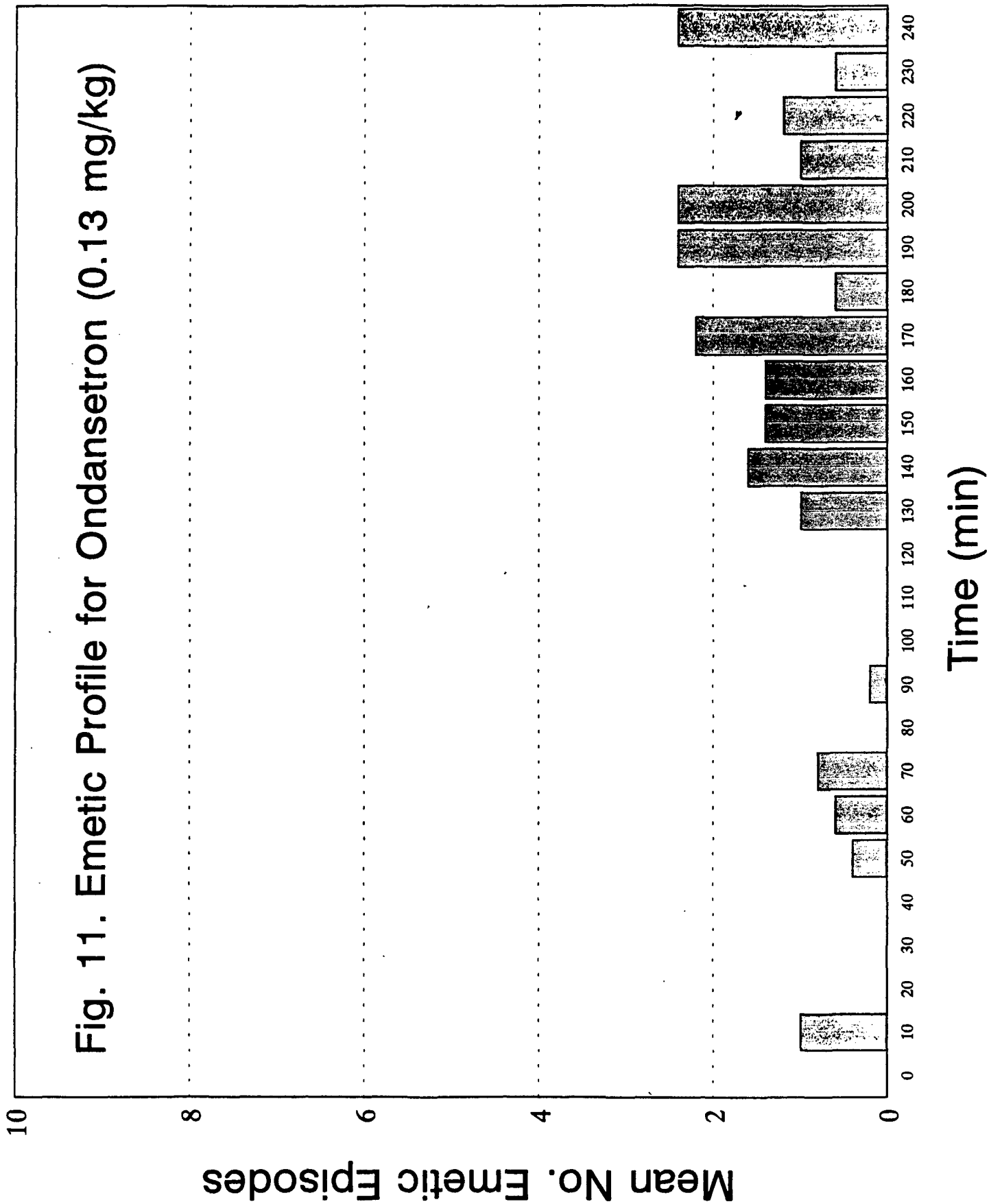
Fig. 11. Emetic Profile for Ondansetron (0.13 mg/kg)
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6
8
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Time (min)
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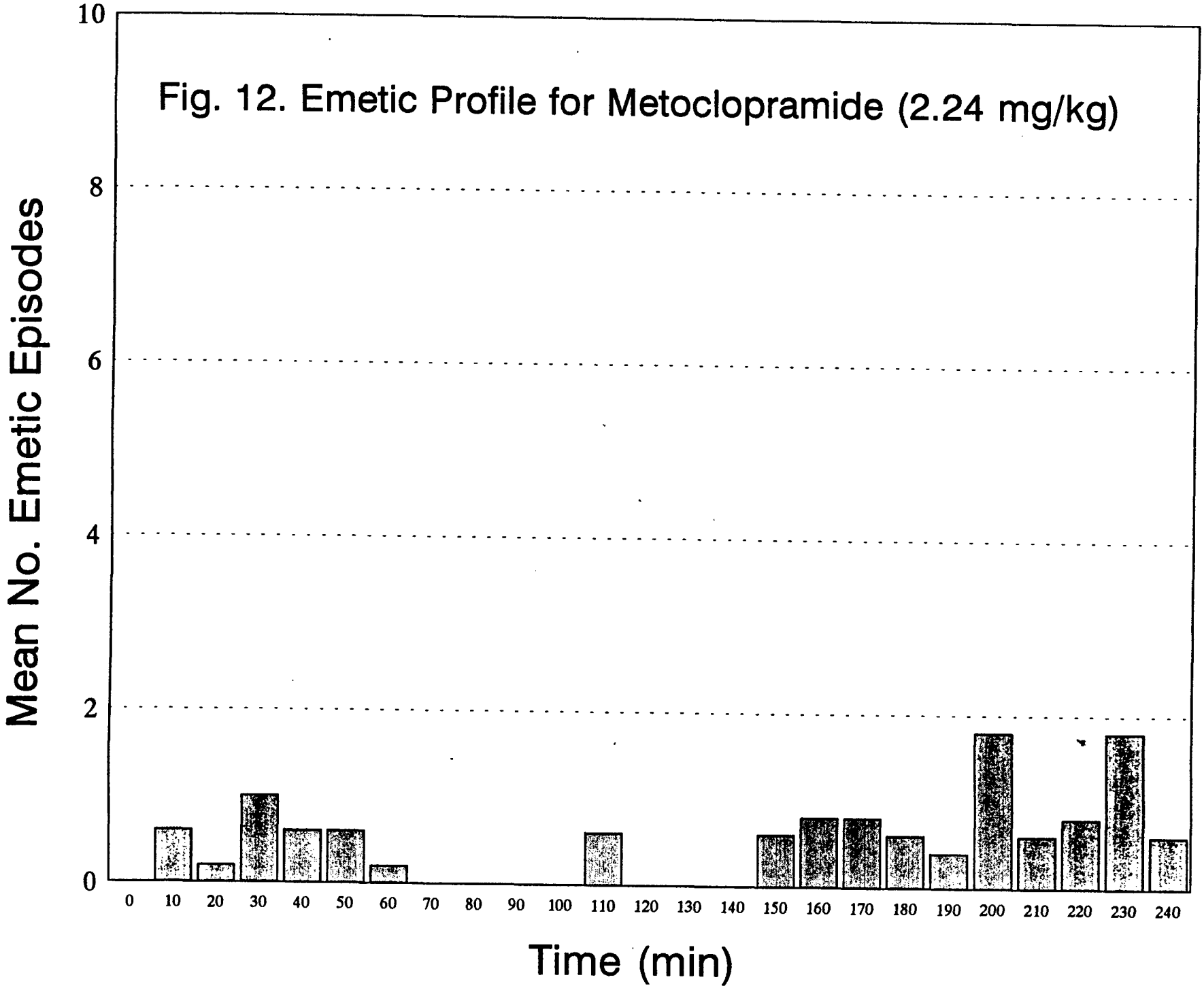
Fig. 12. Emetic Profile for Metoclopramide (2.24 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

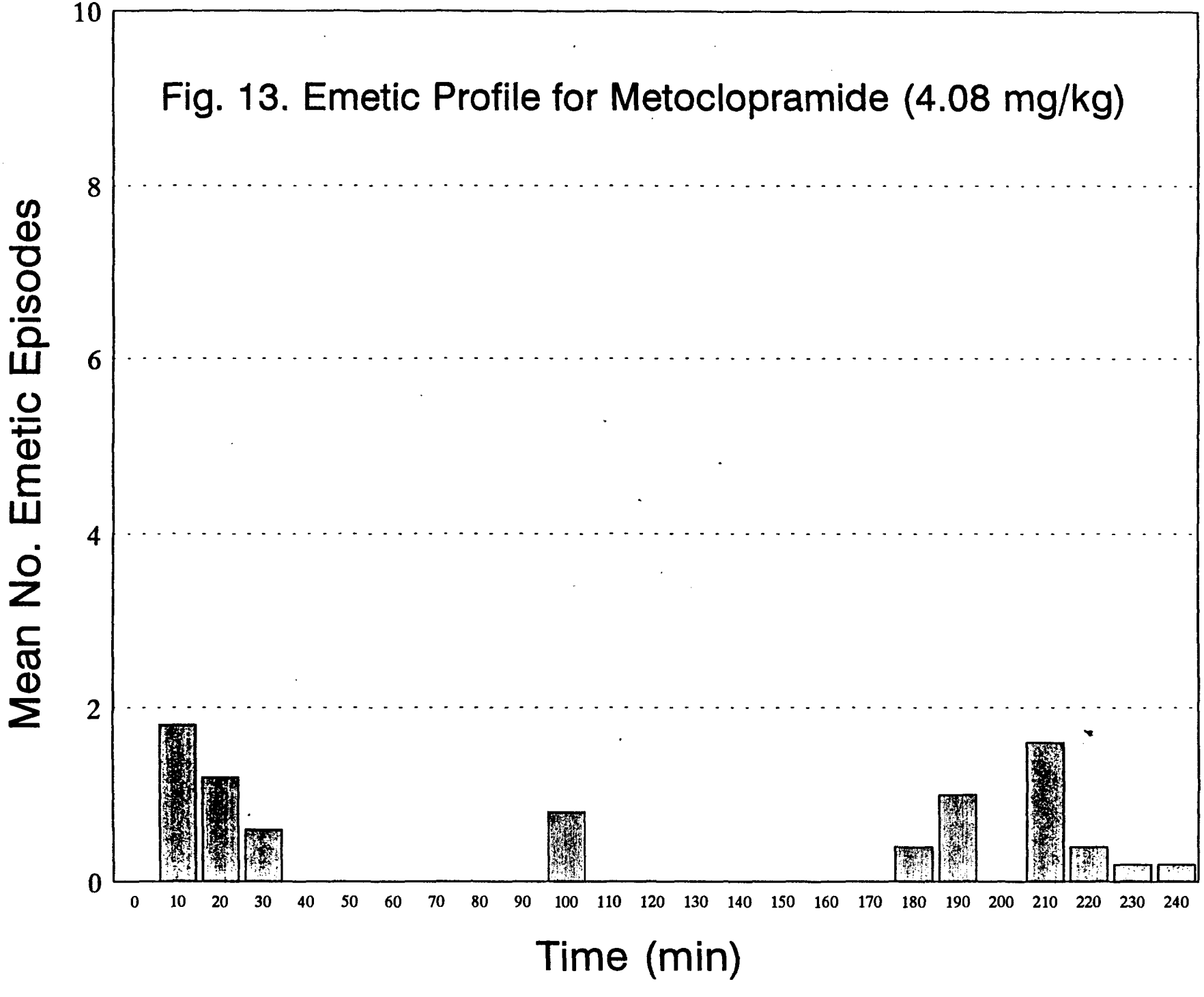
Fig. 13. Emetic Profile for Metoclopramide (4.08 mg/kg)
Mean No. Emetic Episodes
0
2
4
6
8
10
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Time (min)

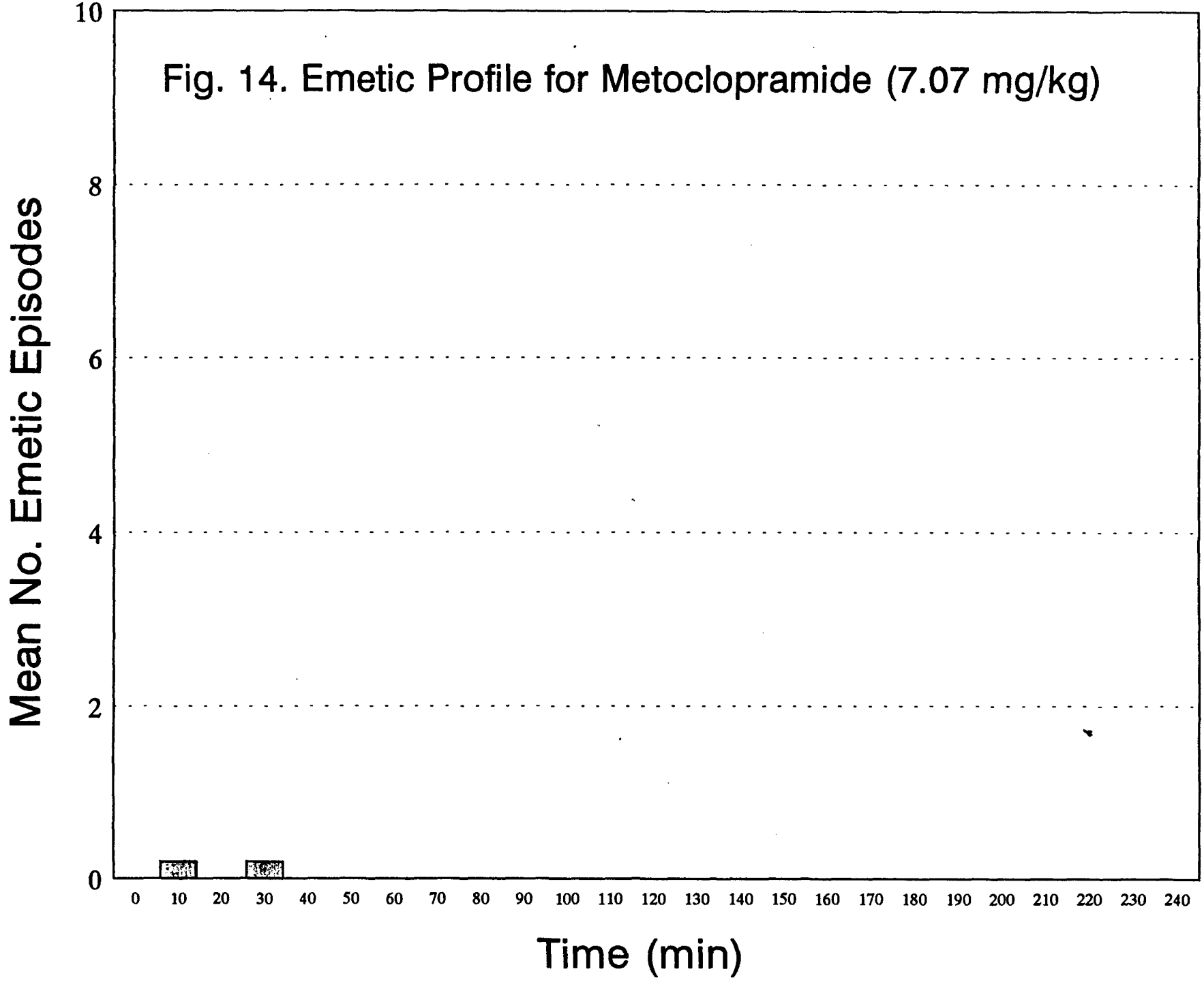
Fig. 14. Emetic Profile for Metoclopramide (7.07 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

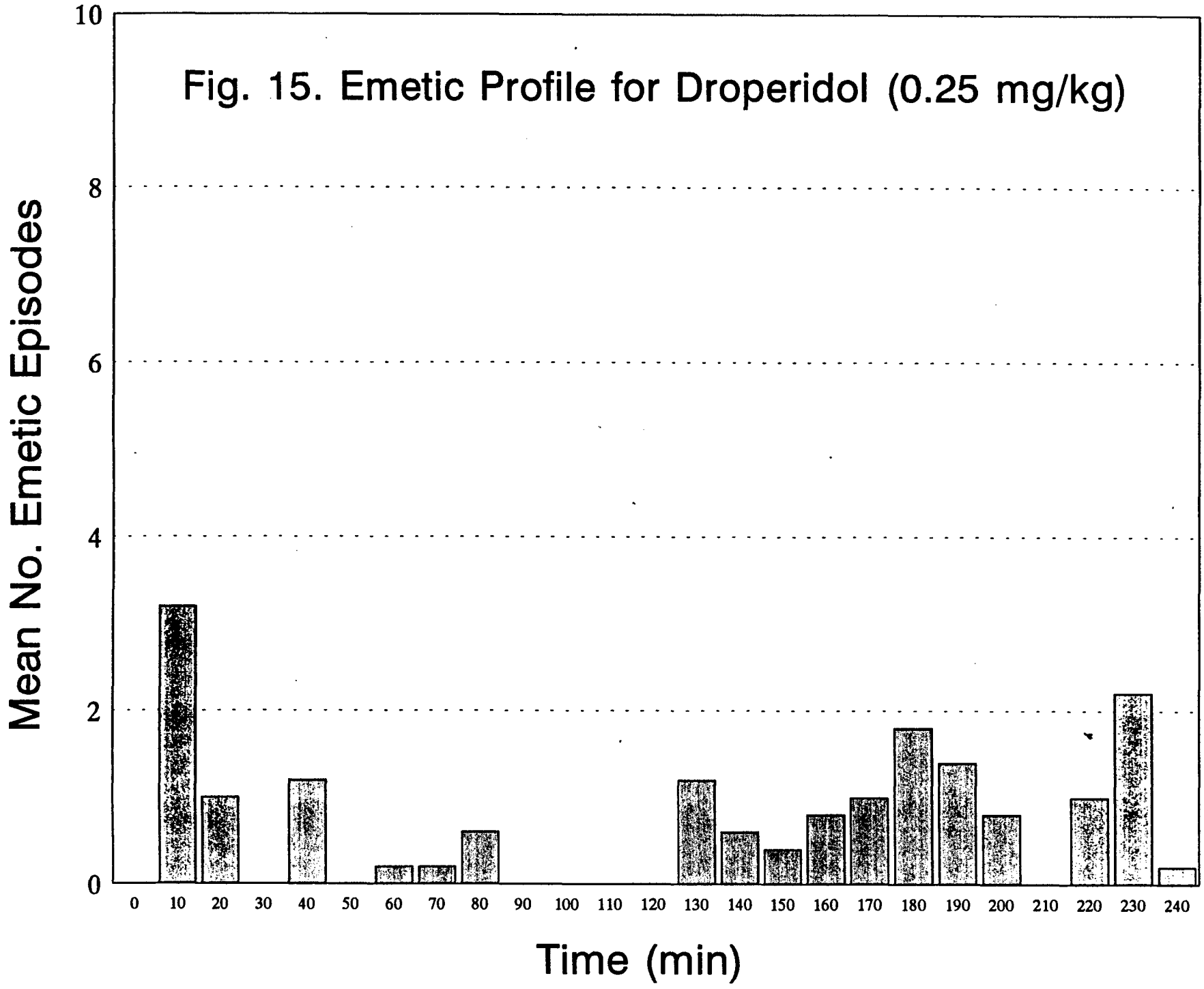
Fig. 15. Emetic Profile for Droperidol (0.25 mg/kg)
Mean No. Emetic Episodes
10
8
6
4
2
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

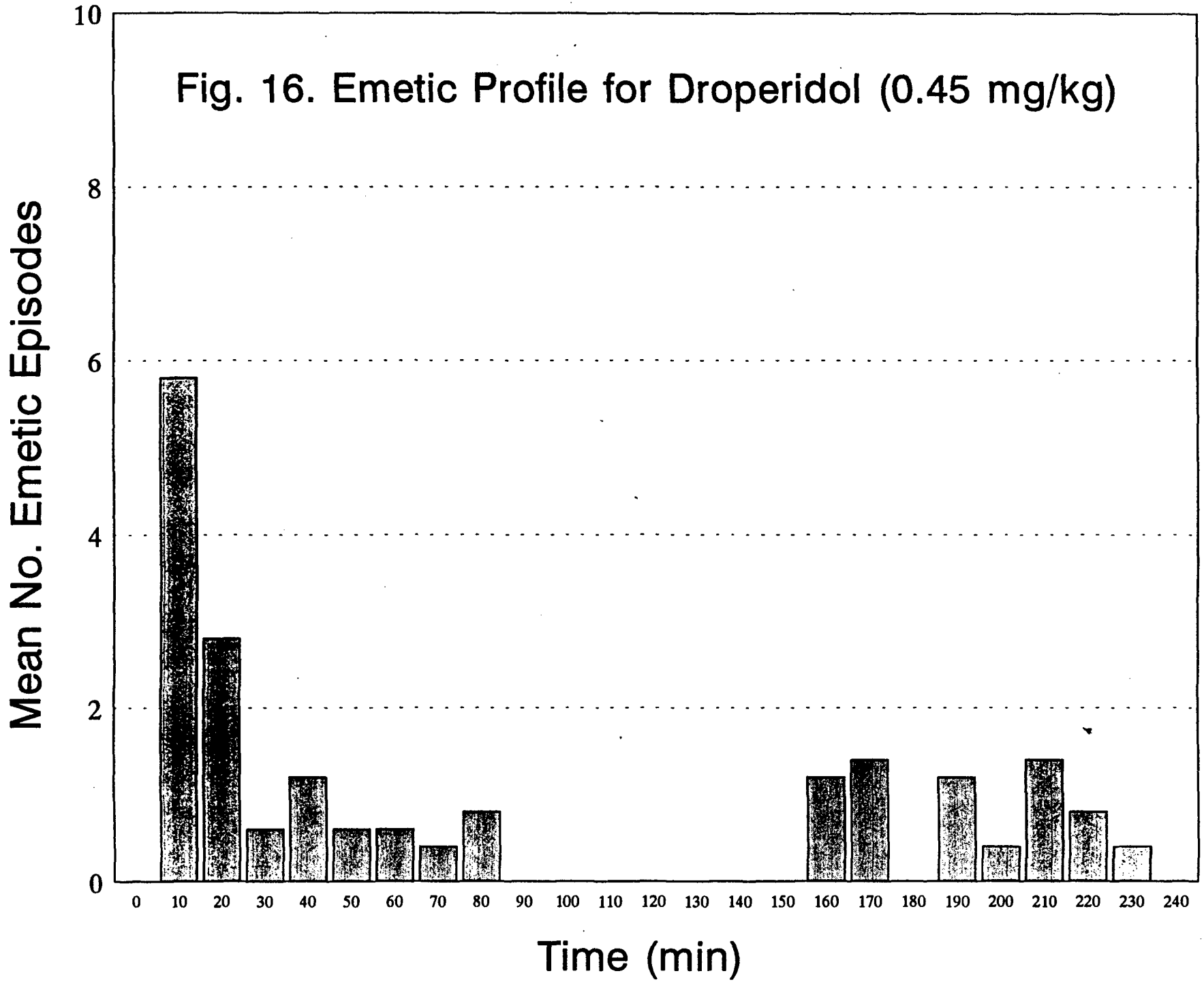
Fig. 16. Emetic Profile for Droperidol (0.45 mg/kg)
Mean No. Emetic Episodes
0
2
4
6
8
10
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

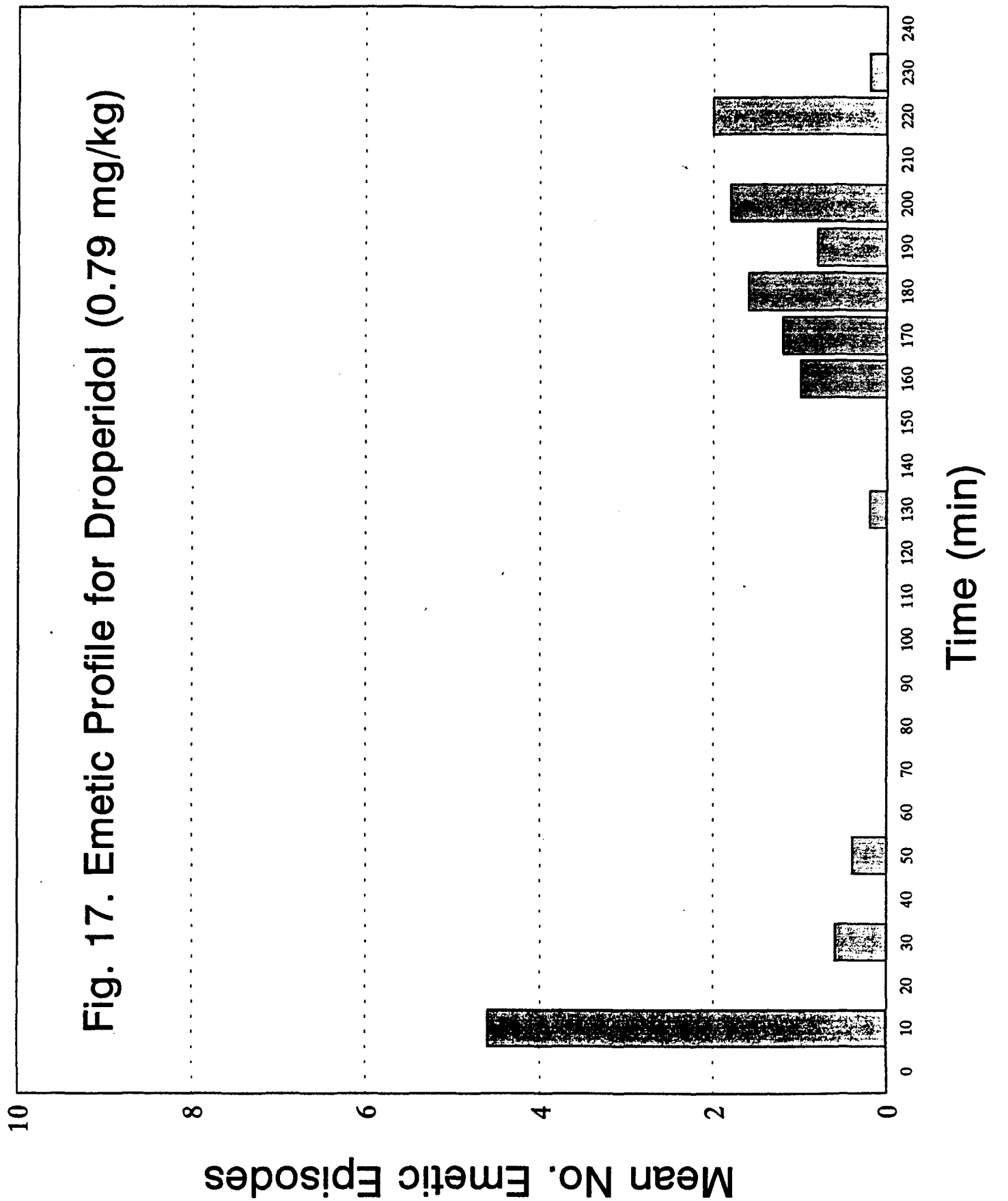
Fig. 17. Emetic Profile for Droperidol (0.79 mg/kg)
Mean No. Emetic Episodes
0
2
4
6
8
10
Time (min)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240

Table 4. Combination of Electroacupuncture and Antiemetic Drug Therapy Against Cyclophosphamide in Ferrets

EA + Drug (mg/kg) *(100 Hz, 1.5V, 10 min)*	*N*	*Mean±S.E. Emetic Episodes* *First Phase[1]*	*Second Phase[2]*
Vehicle	8	18.6±3.9	4.7±1.2
EA + Ondansetron (0.02 mg/kg)	6	14.8±3.4	10.8±2.3*
EA + Ondansetron (0.04 mg/kg)	6	1.7±1.1**	8.7±1.8
EA + Metoclopramide (1.26 mg/kg)	6	6.5±1.1*	5.3±1.7
EA + Metoclopramide (2.24 mg/kg)	6	0.7±0.5**	5.3±1.5
EA + Droperidol (0.25 mg/kg)	6	7.0±1.3*	6.2±1.3
EA + Droperidol (0.45 mg/kg)	6	1.7±0.9**	12.2±1.4**

(*$p \leq 0.05$, **$p \leq 0.005$ compared to vehicle control values; [1] Time 0 to 70 min; [2] Time 130 to 240 min)

CONCLUSIONS

The present study showed that cyclophosphamide produced a biphasic emetic response in the ferret. EA (100 Hz, 1.5V, 10 min) was effective in treating the first emetic phase induced by cyclophosphamide. It had an effect similar to the antiemetic drug ondansetron which also treats the first phase (increases the second phase). Combination therapy of EA and an antiemetic drug (ondansetron 0.04 mg/kg; metoclopramide 2.24 mg/kg) produced the most beneficial effect by significantly reducing the total number of emetic episodes as compared to either treatment alone. Although this was shown to be true for the suboptimal 0.04-mg/kg ondansetron and 2.24-mg/kg metoclopramide dose levels, there is the possibility of no additional antiemetic benefit over that of drug therapy alone at the more effective drug dose levels. However, the results suggested that at lower drug dose levels, EA would be useful as an adjunctive therapy in the treatment of chemotherapy-induced emesis. The results also led to a decrease in the variables evaluated (the number of parameters of EA tested and the number of doses of antiemetic drugs used were sufficient) which decreased a few groups of animals used for this protocol. Acupuncture alone was also examined in which there was no significant adverse effects observed. The significance of this study is that acupuncture as an adjunctive therapy may lead to a decrease in the dose and side effects of the antiemetic drugs which may improve the quality of life for the breast cancer patient. Modern antiemetics have proven to be of use in the prevention of chemotherapy induced N/V; however, a subgroup of patients continues to have N/V. Future clinical studies are necessary to evaluate acupuncture as an adjunctive therapy for the treatment of nausea and vomiting in the breast cancer patient.

REFERENCES

Aglietti, L., Roila, F., Tonato, M., Basurto, C., Bracarda, S., Picciafuoco, M., Ballatori, E., & DelFavero, A. (1990). A Pilot Study of Metoclopramide, Dexamethasone, Diphenylhydramine and Acupuncture in Women Treated with Cisplatin. Can. Chemoth. Pharmacol., 26, 239-240.

Andrews, P. L. R., Rapeport, W.G., and Sanger, G.J. (1988). Neuropharmacology of Emesis Induced by Anti-Cancer Therapy. Trends Pharmac. Sci., 9, 334-341.

Clavel, M., Soukop, M., & Greenstreet, Y. L. A. (1993). Improved Control of Emesis and Quality of Life With Ondansetron in Breast Cancer. Oncology, 50, 180-185.

Coates, A., Abraham, S., Kaye, S. B., Sowerbutts, T., Frewin, C., Fox, R. M., & Tattersall, M. H. N. (1983). On the Receiving End--patient Perception of the Side Effects of Cancer chemotherapy. Eur. J. Can. Clin. Oncol., 19, 203-208.

Cubeddu, L. X., Hoffman, I. S., Fuenmayor, N. T., & Finn, A. L. (1990a). Antagonism of Serotonin S3 Receptors With Ondansetron Prevents Nausea and Emesis Induced by cyclophosphamide-containing Chemotherapy Regimens. J. Clin. Oncol., 8(10), 1721-1727.

Cubeddu, L. X., Hoffmann, I. S., Fuenmayor, N. T., & Finn, A. L. (1990b). Efficacy of Ondansetron (GR 38032F) and the Role of Serotonin in Cisplatin-induced Nausea and Vomiting. New Eng. J. Med., 322, 810-816.

D'Olimpia, S. T., Camocho, F., Chandra, P., Lesser, M., Maldonado, M., Wollner, D., & and Wiernik, P. H. (1985). Antiemetic Efficacy of High-dose Dexamethasone Versus Placebo in Patients Receiving Cisplatin-based Chemotherapy: a Randomized Double-blind Controlled Clinical trial. J. Clin. Oncol., 3, 1133-1135.

Dundee, J. W. (1991). Positive Evidence for P6 Acupuncture Antiemesis. Post. Med. J., 67, 417-422.

Dundee, J. W., Ghaly, R. G., Fitzpatrick, K. T. J., Abram, W. P., & Lynch, G. A. (1989). Acupuncture Prophylaxis of Cancer Chemotherapy-induced Sickness. Journal of Royal Society of Medicine, 82, 268-271.

Dundee, J. W., & Yang, J. (1990). Prolongation of the Antiemetic Action of P6 Acupuncture by Acupressure in Patients Having Cancer Chemotherapy. J. Roy. Soc. Med., 83, 360-361.

Dundee, J. W., Yang, J., & McMillan, C. (1991). Noninvasive Stimulation of the P6 (Neiguan) Antiemetic Acupuncture Point in Cancer Chemotherapy. J. Roy. Soc. Med., 64.

Einhorn, L. H., Nagy, C., Werner, K., & Finn, A. L. (1990). Ondansetron: A New Antiemetic for Patients Receiving Cisplatin Chemotherapy. J. Clin. Oncol., 8(4), 731-735.

Fraschini, G., Ciociola, A., Esparza, L., Templeton, D., Holmes, F. A., Walters, R. S., & Hortobagyi, G. N. (1991). Evaluation of Three Oral Dosages of Ondansetron in the Prevention of Nausea and Emesis Associated With Cyclophosphamide-doxorubicin Chemotherapy. J. Clin. Oncol., 9(7), 1268-1274.

Hawthorn, J., Ostler, K. J., & Andrews, P. L. R. (1988). The role of the Abdominal Visceral Innervation and 5-hydroxytryptamine M-receptors in Vomiting Induced by the Cytotoxic drugs Cyclophosphamide and Cis-platin in the Ferret. Q. J. Exp. Physiol., 73, 7-21.

Hesketh, P. J., & Gandara, D. R. (1991). Serotonin Antagonists: A New Class of Antiemetic Agents. J. Nat. Canc. Inst., 83(9), 613-620.

Kent, G., & Brondum, J. (1988). A Large Outbreak of Acupuncture Associated Hepatitis B. Am J of Epidemiology, 127, 591-8.

Lao, L., Wong, R. H., Berman, B., & Wynn, R. L. (1995). Electroacupuncture Reduces Morphine-Induced Emesis in Ferrets: A Pilot Study. J. Altern. Complem. Med., 1(3), 1-4.

O'Connor, J., & Bensky, D. (Ed.). (1981). Acupuncture: a Comprehensive Text. Chicago: Eastland Press.

Rosso, R., Campora, E., Cetto, G., Fosser, V., Marangolo, M., & Oliva, C. (1991). Oral Ondansetron (GR 38032F) for the Control of Acute and Delayed Cyclophosphamide-induced Emesis. Anticancer Res., 11, 937-940.

Sanger, G. J. (1990). New Antiemetic Drugs. Can. J. Physiol. Pharmacol., 68, 314-324.
Stux, G., & Pomeranz, B. (1987). History of Acupuncture. In Acupuncture: textbook and atlas (pp. 35). Berlin: Springer-Verlag.

Watcha, M. F., & White, P. F. (1992). Postoperative Nausea and Vomiting. Anesthesiology, 77(1), 162-184.

Wong, R. H., Lao, L., Berman, B., Carter, A., & Wynn, R. L. (1996). Biphasic Emetic Response of Cyclophosphamide in the Ferret. Pharmacol. Biochem. Behav., Under review.

Wright, R. S., Kupperman, J. I., & Liebhaber, M. I. (1991). Bilateral Tension Pneumothoraces After Acupuncture. Western Journal of Medicine, 154(1), 102-103.

Wynn, R. L., Ebakuwa, E., & Thut, P. D. (1993). The Effects of Different Antiemetic Agents on Morphine-Induced Emesis in Ferrets. Europ. J. Pharmacol., 241, 47-54.

APPENDIX

(Manuscript Under Review)

WONG, R.H., L. LAO, B.M. BERMAN, A.K. CARTER, AND R.L. WYNN. *Biphasic emetic response of cyclophosphamide in the ferret.* PHARMACOL BIOCHEM BEHAV.
Cyclophosphamide (177 mg/kg i.v., n=8) produced a biphasic emetic response in the ferret with a mean (±S.E.) of 23.3±4.0 emetic episodes during a four hour observation period. The emetic profile of cyclophosphamide showed a first phase with 18.6±3.9 episodes and a second phase with 4.7±1.2 episodes. Ondansetron (0.07 and 0.13 mg/kg i.v.) and droperidol (0.25 and 0.79 mg/kg i.v.) significantly reduced the number of emetic episodes in the first phase. Metoclopramide (2.24, 4.08, and 7.07 mg/kg i.v.) also significantly reduced the number of emetic episodes in the first phase and the dose of 7.07 mg/kg completely prevented emetic episodes in the second phase. In addition, ondansetron treated ferrets (0.04, 0.07, and 0.13 mg/kg i.v.) had a significant increase in the number of emetic episodes in the second phase.

Keywords: Cyclophosphamide, emesis, ondansetron, metoclopramide, droperidol, ferret

Breast cancer patients are often treated with an adjuvant chemotherapy regimen which includes cyclophosphamide (2,3,8). This alkylating agent has a high potential for inducing nausea and vomiting which adds to the difficult nature of cancer treatment (5,8). Unlike many chemotherapy agents, emesis associated with cyclophosphamide may often extend up to 72 hours (2). Cyclophosphamide induces emesis in a ferret model (1,6) possibly through release of serotonin to stimulate the 5-HT_3 receptor in the gastrointestinal tract and the chemoreceptor trigger zone (5,6). The serotonin (5-HT_3) receptor antagonists have been shown to be effective antiemetics for cyclophosphamide-induced emesis in ferrets (1) and humans (2,3,5,8). Side effects have included headache, light-headedness and transient elevations of hepatic transaminases

(2,3,4,5,7,8). Metoclopramide, a dopamine (D_2) receptor/5-HT_3 receptor antagonist, has been moderately effective in reducing cyclophosphamide-induced emesis in humans (2). However, metoclopramide has been shown to produce adverse extrapyramidal reactions in humans (9). The dopamine (D_2) antagonist droperidol has not been tested against cyclophosphamide-induced emesis. The purpose of this study was to further examine the characteristics of the emetic effects of intravenous cyclophosphamide in the ferret and to evaluate the antiemetic efficacy of ondansetron, a selective 5-HT_3 antagonist, metoclopramide, and droperidol.

MATERIAL AND METHODS

Ferrets (fitch or albino) were castrated males, 1.0-2.0 kg in weight (Triple F Farm, Sayre, PA), and were housed three to a cage on a twelve hour light cycle. Food (Lab Diet) and water were given ad libitum. Ferrets were used only one time. For testing, ferrets were placed under general anesthesia (isoflurane 5%-O_2 mixture) delivered from a vaporizer (Fortec), calibrated for isoflurane, through polyethylene tubing into an anesthesia chamber. The anesthetic was scavenged out using a vacuum tubing vented to the outside air. Each ferret was removed after loss of righting (2-5 min) and immediately weighed. For the intravenous (i.v.) injections, each animal was maintained under general anesthesia (isoflurane 2.5%-O_2) with a second vaporizer (Fortec) through a small nose cone. Both forepaws were shaved for ease of vein location. Cyclophosphamide monohydrate (Sigma) was dissolved in a small amount of absolute alcohol (200 mg/400 microliters) immediately prior to injection. Saline (154 mM) was used to dilute the cyclophosphamide to a final concentration of 100 mg/mL. Cyclophosphamide injections were made into the cephalic vein on the dorsal aspect of a front paw using a rubber tourniquet and a 3 or 5 ml syringe with a 25 G needle. Intravenous puncture was confirmed by aspiration of a small

volume of blood into the syringe, and the injections were confirmed by the lack of resistance to the syringe plunger. The following log doses of cyclophosphamide were tested: 56 (n=6), 100 (n=6), 177 (n=8), and 237 (n=2) mg/kg. For administration of the antiemetic drugs (n=5/dose), a second i.v. injection was made into the opposite forepaw within one minute following cyclophosphamide (177 mg/kg) injection. This dose of i.v. cyclophosphamide was chosen for the subsequent antiemetic experiments since it produced the maximal number of emetic episodes without toxicity. Ondansetron (Glaxo, 2 mg/mL), metoclopramide (A.H. Robbins, 5 mg/mL), and droperidol (American Regent, 2.5 mg/mL) were obtained from the University of Maryland Hospital pharmacy as the commercial preparations. The doses of the antiemetic drugs were given as follows: ondansetron, 0.04, 0.07, 0.13 mg/kg; metoclopramide, 2.24, 4.08, 7.07 mg/kg; and droperidol, 0.25, 0.45, 0.79 mg/kg.

After injection, each ferret was then placed into an individual compartment (60 x 60 x 38 cm^2) of a six-compartment cage rack with wire mesh floors elevated to the height of the door threshold (modified with a plexiglass front door) for observation. Each ferret was observed for four hours after recovering from anesthesia (3-10 min). The time and number of each episode of retching and vomiting were recorded. Retching was counted as the rhythmic contraction of the abdomen without expulsion of material, and emesis as a contraction with the expulsion of solid or liquid. Total emetic episodes were averaged for each group ($\pm$S.E.) and the effect of treatment was calculated as the percent reduction of emetic episodes as previously described (10). Differences between the mean number of emetic episodes for the treatment groups compared to the cyclophosphamide group (177 mg/kg) were compared by Student's two-tail t-test with a $p \leq 0.05$ considered significant. This study was approved by the Institutional Animal Care and Use

Committees at the School of Medicine and the Dental School, University of Maryland at Baltimore.

RESULTS

The dose effect profile of cyclophosphamide-induced emesis in the ferret after intravenous injection is shown in Table 1. The dose of 237 mg/kg produced oedema around the eyes and erythema in the facial area. This was interpreted as toxicity and only two animals were tested at this dose. Evaluation of the duration of emetic episodes using time bins of 10 min revealed two distinct phases over the entire duration of effect for the doses of 100 and 177 mg/kg. These two emetic phases for the dose of 177 mg/kg are shown in Figure 1. The first phase had a mean onset time of 1.1±0.5 min and duration of 70 min. The second phase occurred 60 min after the termination of the first phase.

On the basis of the total number of emetic episodes, ondansetron reduced cyclophosphamide-induced emesis (177 mg/kg) by 0, 42, and 9% (0.04, 0.07, 0.13 mg/kg), metoclopramide by 48, 65 and 98% (2.24, 4.08, 7.07 mg/kg), and droperidol by 24, 16, and 38% (0.25, 0.45, 0.79 mg/kg). Significant differences in the mean number of total emetic episodes were found for metoclopramide at doses of 4.08 mg/kg ($p \leq 0.05$) and 7.07 mg/kg ($p \leq 0.005$) compared to vehicle control. All three antiemetic drugs (ondansetron at 0.07 and 0.13 mg/kg; metoclopramide at 2.24, 4.08, and 7.07 mg/kg; and droperidol at 0.25 and 0.79 mg/kg) significantly reduced the number of emetic episodes in the first phase (Table 2). Metoclopramide at 7.07 mg/kg completely prevented the second emetic phase, whereas both ondansetron and droperidol treated ferrets had a significant increase in the number of emetic episodes in this phase (Table 2).

DISCUSSION

A previous literature report evaluating cyclophosphamide at two dosages and various routes of administration showed that this chemotherapeutic drug produced emesis in the ferret (6). At an i.v. dose of 100 mg/kg, this study reported a mean number (±S.E.) of 0.4±0.2 emetic episodes and 4.6±2.4 retches during a four hour observation period (6). However, in our study, we observed 7.3±3.2 emetic episodes and 30.5±17.5 retches at 100 mg/kg (i.v.) with a four hour observation period (Table 1). The only methodological differences were in our use of direct i.v injection and in our preparation of the cyclophosphamide, using a final concentration of 100 mg/mL which is commonly used in chemotherapy patients. In our study, a dose of 177 mg/kg produced two phases of emesis (Fig. 1).

The early onset emetic phase induced by cyclophosphamide may possibly be due to direct central effects involving the 5-HT_3 or D_2 receptor. The antiemetic drugs ondansetron (a 5-HT receptor antagonist) and droperidol (D_2 receptor antagonist) were only able to significantly reduce the number of emetic episodes in this phase while increasing the second phase (Table 2). Furthermore, the emetic profile of cyclophosphamide for the first phase (Fig. 1) showed a decline in episodes over time which may be indicative of the clearance of the drug from the site of action.

The second emetic phase induced by cyclophosphamide may involve a different mechanism. Metoclopramide, a D_2/5-HT_3 antagonist, was the only effective antiemetic drug that could reduce the number of emetic episodes in this phase (Table 2). However, the high dose of metoclopramide (7.07 mg/kg) did produce adverse central nervous system effects (eg. mean±S.E. of 82.6±16.6 head shakes as compared to control, 19.3±5.9). The significant increase in emetic episodes in the second phase seen with ondansetron and droperidol (Table 2) may be due to the elimination of these drugs in the tissues, thus allowing for the emetogenic effects of cyclophosphamide to predominate. Further

studies are necessary to examine the different mechanisms that may be involved in the biphasic emetogenic response of cyclophosphamide. Since patients may vomit for at least two days (or more) after cyclophosphamide, future studies in the ferret will also need to utilize longer observation periods in order to evaluate similarities with what occurs in patients.

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REFERENCES

1. Andrews, P.L.R.; Rapeport, W.G.; Sanger, G.J. Neuropharmacology of emesis induced by anti-cancer therapy. Trends Pharmacol. Sci. 9: 334-341; 1988.

2. Clavel, M.; Soukop, M.; Greenstreet, Y.L.A. Improved control of emesis and quality of life with ondansetron in breast cancer. Oncology 50: 180-185; 1993.

3. Cubeddu, L.X.; Hoffman, I.S.; Fuenmayor, N.T.; Finn, A.L. Antagonism of serotonin S3 receptors with ondansetron prevents nausea and emesis induced by cyclophosphamide-containing chemotherapy regimens. J. Clin. Oncol. 8(10): 1721-1727; 1990.

4. Einhorn, L.H.; Nagy, C.; Werner, K.; Finn, A. L. Ondansetron: A new antiemetic for patients receiving cisplatin chemotherapy. J. Clin. Oncol. 8(4): 731-735; 1990.

5. Fraschini, G.; Ciociola, A.; Esparza, L.; Templeton, D.; Holmes, F.A.; Walters, R.S.; Hortobagyi, G.N. Evaluation of three oral dosages of ondansetron in the prevention of nausea and emesis associated with cyclophosphamide-doxorubicin chemotherapy. J. Clin. Oncol. 9(7): 1268-1274; 1991.

6. Hawthorn, J.; Ostler, K.J.; Andrews, P.L.R. The role of the abdominal visceral innervation and 5-hydroxytryptamine M-receptors in vomiting induced by the cytotoxic drugs cyclophosphamide and cis-platin in the ferret. Q. J. Exp. Physiol. 73: 7-21; 1988.

7. Hesketh, P.J.; Gandara, D.R. Serotonin antagonists: A new class of antiemetic agents. J. Nat. Canc. Inst. 83(9): 613-620; 1991.

8. Rosso, R.; Campora, E.; Cetto, G.; Fosser, V.; Marangolo, M.; Oliva, C. Oral ondansetron (GR 38032F) for the control of acute and delayed cyclophosphamide-induced emesis. Anticancer Res. 11: 937-940; 1991.

9. Sanger, G.J. New antiemetic drugs. Can. J. Physiol. Pharmacol. 68: 314-324; 1990.

10. Wynn, R.L.; Ebakuwa, E.; Thut, P.D. The effects of different antiemetic agents on morphine-induced emesis in ferrets. Europ. J. Pharmacol. 241: 47-54; 1993.

Table 1. Emetogenic Effect of Cyclophosphamide by Dose in Ferrets

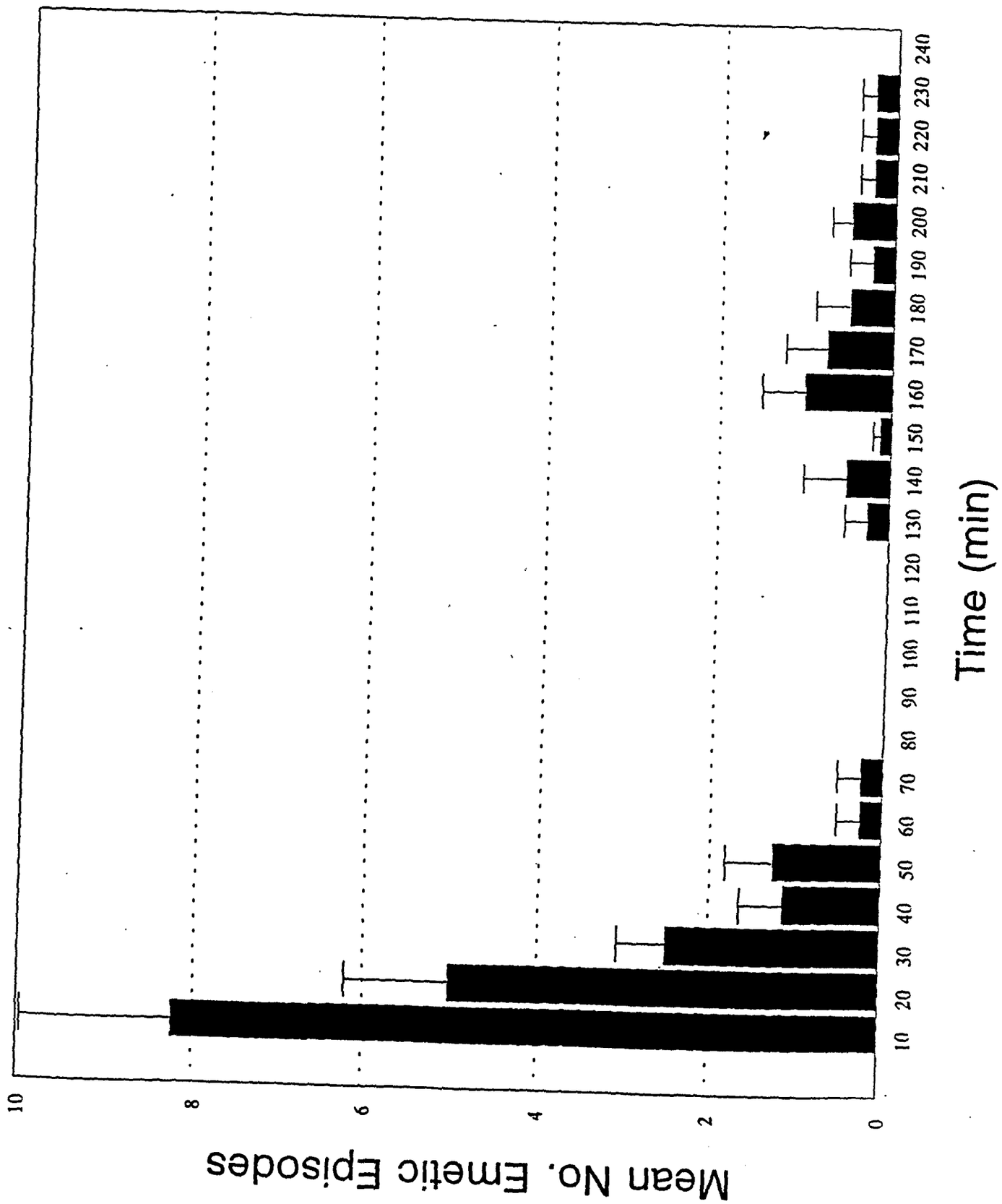
Dose (mg/kg)	*No. Vomiting/N*	*Mean±S.E. Emetic Episodes*	*Mean±S.E. Retches*
Cyclophosphamide			
56	4/6	2.2±0.9	2.8±1.9
100	5/6	7.3±3.2	30.5±17.5
177	8/8	23.3±4.0	85.3±20.4
237	2/2	23.5±7.5	62.5±38.5

Table 2. Dose Response of Antiemetics Against Cyclophosphamide in Ferrets

Dose (mg/kg)	*N*	*Mean±S.E. Emetic Episodes*	
		First Phase[1]	*Second Phase*[2]
Vehicle	8	18.6±3.9	4.7±1.2
Ondansetron			
0.04	5	12.2±3.7	15.4±2.8**
0.07	5	0.4±0.4**	11.0±2.1*
0.13	5	2.8±1.7*	18.2±3.5**
Metoclopramide			
2.24	5	3.2±2.7*	8.2±3.1
4.08	5	3.6±1.9*	3.8±1.3
7.07	5	0.4±0.2**	0.0±0.0
Droperidol			
0.25	5	5.8±2.6*	11.4±2.7*
0.45	5	12.0±2.3	6.8±2.2
0.79	5	5.6±3.1*	8.8±1.7

(*p≤0.05, **p≤0.005 compared to vehicle control values; [1] Time 0 to 70 min; [2] Time 130 to 240 min)

Fig. 1. Emetic profile of i.v. cyclophosphamide at 177 mg/kg. Two distinct phases of emesis are present.


Mean No. Emetic Episodes
0
2
4
6
8
10
Time (min)
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240

BIBLIOGRAPHY

Abstracts:

Wynn, R.L., Carter, A., Meszler, R., Lao, L., Berman, B.M., and Wong, R.H., Dose-Response of Cyclophosphamide-Induced Emesis in the Ferret, Presentation at the Society for Neuroscience, San Diego, California, November, 1995.

Lao, L., Use of Acupuncture in Small Mammals, For presentation at the Eighth Small Mammals Veterinary Conference, Baltimore, Maryland, August, 1996.

Publications:

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DOSE-RESPONSE OF CYCLOPHOSPHAMIDE-INDUCED EMESIS IN THE FERRET. R.L. Wynn, A. Carter, R. Meszler*, L. Lao, B.M. Berman and R.H. Wong. Department of Family Medicine, School of Medicine, and Departments of Anatomy and Pharmacology, Dental School, University of Maryland, Baltimore, MD 21201

Nausea and vomiting are severe side-effects often associated with cancer chemotherapy and may affect treatment decisions. Cyclophosphamide is a commonly used chemotherapy agent for breast cancer and induces emesis in the ferret. In order to examine the emetogenic effect of cyclophosphamide, ferrets (1.0-1.8 kg) were placed under general anesthesia (isoflurane 5%-O_2 mixture) and were administered logarithmic doses of i.v. cyclophosphamide. The mean number (±SE) of emetic episodes and retches were: 2.2±0.9 episodes and 2.8±1.9 retches at 56 mg/kg, 7.3±3.2 and 30.5±17.5 at 100 mg/kg, 23.3±4.0 and 85.3±20.4 at 177 mg/kg, and 23.5±7.5 and 62.5±38.5 at 237 mg/kg. In addition, various antiemetics were given i.v. immediately following cyclophosphamide injection. Ondansetron reduced emetic episodes by 0% and 43% (0.04 and 0.07 mg/kg), metoclopramide by 65% and 98% (4.08 and 7.07 mg/kg), and droperidol by 16% and 24% (0.45 and 0.25 mg/kg). These results indicate that cyclophosphamide induces emesis in a dose-dependent manner and may be useful in evaluating conventional and complementary therapies for the treatment of chemotherapy-induced emesis.

Support provided by the U.S. Army Breast Cancer Research Program #DAMD17-94-J-4325.

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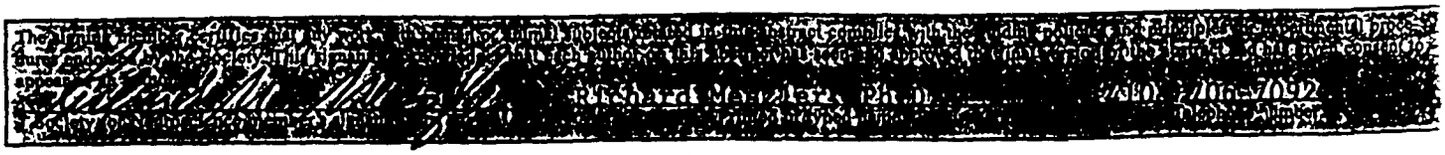

ABSTRACT

SESSION TITLE: USE OF ACUPUNCTURE IN SMALL MAMMALS

Lixing Lao, Ph.D.

Nausea and vomiting are severe side-effects often associated with cancer chemotherapy and may affect treatment decisions. Cyclophosphamide is a commonly used chemotherapy agent for breast cancer and induces emesis in the ferret. In order to examine the effects of electroacupuncture (EA) on the emetogenic effect of cyclophosphamide, ferrets (1.0-1.8 kg) were placed under general anesthesia (isoflurane 5%-O_2 mixture) and were administered logarithmic doses of i.v. cyclophosphamide. A dose of 177 mg/kg produced the maximal number of emetic episodes (23.3 ± 4.0 episodes) with an emetic profile consisting of two phases (first phase 18.6 ± 3.9 episodes; second phase 4.7 ± 1.2 episodes). For treatment, EA was given under general anesthesia followed by i.v. cyclophosphamide (177 mg/kg). Various parameters were evaluated and the results indicated that EA (100 Hz, 1.5V, 10 min) effectively treated the first emetic phase induced by cyclophosphamide (9.3 ± 1.8 episodes for the first phase). EA had an effect similar to the antiemetic drug ondansetron which also treated the first phase. Preliminary studies using combination therapy of EA and metoclopramide (2.24 mg/kg) showed a significant reduction in the number of emetic episodes ($p \leq 0.005$). This study indicates that EA would be useful as an adjunctive therapy in the treatment of chemotherapy-induced emesis in the ferret.

[approx. 200 words]

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